1

IMMUNE PROTECTION FROM LETHAL MURINE CYTOMEGALOVIRUS INFECTION FOLLOWING LETHAL DOSE IRRADIATION AND SYNGENEIC COTRANSPLANTATION OF EITHER COMMON LYMPHOCYTE PROGENITORS OR LYMPH NODE CELLS WITH HEMATOPOIETIC STEM CELLS.

Brown JM, BitMansour A, Mocarski ES, Akashi K, Weissman IL, Stanford University School of Medicine, Stanford, California

The $Lin^{-}IL\text{-}7R^{+}thy\text{-}1^{-}Scal^{10}cKit^{10}$ population from adult mouse bone marrow possesses a rapid lymphoid restricted reconstitution capacity *in vivo*. This study is designed to evaluate the ability of these common lymphocyte progenitor cells (CLP) and unfractionated lymph node (LN) cells to functionally reconstitute immune response to viral challenge. CLP were sorted using IL-7R(expression and conventional markers including Lin, Thy1.1, Sca-1, and cKit. Lethally irradiated (920 rad) C57B1/Ka-Thy1.1 male mice, 8-12 weeks of age, were reconstituted with either (1) 200 syngeneic hematopoietic stem cells (HSC); (2) 200 HSC and 1 x 10^7 LN cells; or (3) 200 HSC and 3000 CLP. These mice were challenged on Day +14 post-transplantation 5.0 x 10^5 plaque forming units *ip* of a *lacZ*-tagged murine cytomegalovirus (MCMV RM427). On Day +35, the peripheral blood of all surviving mice was analyzed using FACS which confirmed the proliferation of T and B cell populations in the mice cotransplanted with CLP. The cotransplantation of CLP or LN cells with HSC conferred a survival advantage following early viral challenge when compared to mice receiving HSC alone ($p<0.09$ and $p<0.037$, respectively).

2

IV BUSULFAN, CYCLOPHOSPHAMIDE (BUCY) AND HEMOPOIETIC STEM CELLS FOR NON-HODGKIN LYMPHOMAS (NHL).

BS Anderson, H Fernandez, P Cagnoni, R Jones, V Gian, J Wingard, S Tarantolo, A Kashyap, SJ Forman, WW Hu, K McWilliams, H Tran, F Cabanillas, RE Champlin, UT MD Anderson Cancer Ctr, Houston TX, U Miami, Miami, FL, U Colorado, Denver, CO, UF Shands Cancer Center, Gainesville, FL, U Nebraska, Omaha, NE, City of Hope Natl. Med. Ctr, Duarte, CA, Stanford U. Medical Center, Palo Alto, CA

Oral Bu and Cy as pretransplant conditioning therapy is not used to a major extent for malignant lymphomas. It carries a high risk for lethal liver toxicity from unpredictable oral Bu bioavailability. We have now designed a parenteral Bu formulation (Busulfex™), which is being used as conditioning therapy prior to stem cell transplantation (HSCT). This is part of an ongoing phase II (autologous and allogeneic) HSCT study with eligibility open to NHL patients. Bu was given at 0.8 mg/kg every 6 hrs χ 16 doses, followed by Cy 60 mg/kg daily for 2 days. As part of this study, we treated 16 NHL patients (9 males/ 7 females), with a median age of 44 years (range 22-60 years). The follow-up of all patients ranged from 3 months to ≥22months. Twelve of the 16 received the treatment as their first transplant. Of these twelve, one was in a second remission and remains in CR at 16 months. Three relapsing patients were untested (1), or had chemotherapy-sensitive disease (2); all three are in clinical CR from 9-22 months. Eight of these twelve patients were either primary refractory, or had a chemotherapy refractory relapse prior to HSCT, and all eight attained a clinical remission. Four of these 8 have suffered progressive disease at 4.5, 9, 9.5 and 15 months, and one patient died from pneumonia and pulmonary hemorrhage at 2.5 mos. One year progression free survival for all patients who had a first transplant is projected to ≥55%. Four of the 16 patients had received a prior transplant, and had active disease at the time of their second transplant. Three of these four patients have relapsed (range 2-22 mos) and one died early (≤1 mo) from Aspergillus pneumonia.

The most common early (3 months) treatment-related toxicity was grade II-III mucositis. Importantly, no patient died from veno-occlusive disease and there was no neurotoxicity recorded. Pharmacokinetic analysis showed significantly less interindividual variations in AUC:s than what is seen with oral busulfan.

We conclude, that this iv Busulfan formulation (Busulfex™) is well tolerated, with an impressive toxicity profile, and we suggest that it should be considered for further testing in pretransplant conditioning therapy prior to HSCT for patients with high-risk Non-Hodgkin lymphomas.

3

TWO-YEAR RESULTS OF A PHASE III STUDY COMPARING TACROLIMUS AND CYCLOSPORINE FOR GVHD PROPHYLAXIS IN UNRELATED DONOR BONE MARROW TRANSPLANTATION.

J. Antin[1], R. Nash[2], C. Karanes[3], J. Fay[4], B. Avalos[5], A. Yeager[6], D. Przepiorka[7], S. Davies[8], F. Petersen[9], C. Anasetti[1], R. Storb[1], V. Ratanatharathorn[10]. [1]Brigham Women's Hospital, Boston, MA. [2]Fred Hutchinson Cancer Research Center, Seattle, WA. [3]Wayne State/Harper Hospital, Detroit, MI. [4]Baylor U Medical Center, Dallas, TX. [5]U of Ohio Columbus, OH. [6]Emory U, Atlanta, GA. [7]Texas Children's Hospital, Houston, TX. [8]U of Minnesota, Minneapolis, MN. [9]U of Utah, Salt Lake City, UT. [10]U of Michigan, Ann Arbor, MI

A total of 180 patients were enrolled in a Phase III randomized trial comparing tacrolimus and cyclosporine in combination with short course methotrexate for the prophylaxis on acute GVHD following unrelated donor bone marrow transplantation from March 1995 through September 1996. Randomization was stratified on the degree of HLA match between donor and recipient (complete match vs. one antigen mismatch). Ninety patients were randomized to each treatment arm. Kaplan-Meier 100 day estimates of acute GVHD (censoring for relapse, death and second transplant) and two year estimates of chronic GVHD, relapse free survival and overall survival are as follows:

	Tacrolimus	Cyclosporine	pValue
Acute GVHD (100 days)	**56%**	**74%**	**0.0002**
Chronic GVHD (2 year)	**76%**	**69%**	**0.96**
Relapse free survival (2 year)	**47%**	**42%**	**0.57**
Overall survival (2 year)	**54%**	**50%**	**0.46**

Chronic GVHD rates between the two groups were comparable, 23 patients and 22 patients in the tacrolimus and cyclosporine arm respectively had limited chronic GVHD while 15 patients and 13 patients had extensive chronic GVHD. These results show that tacrolimus is more effective than cyclosporine for prevention of acute GVHD without an increased risk of relapse or death and show similar chronic GVHD rates.

4

CLINICAL CHARACTERISTICS OF DE NOVO AND QUIESCENT CHRONIC GRAFT-VERSUS-HOST DISEASE IN JAPANESE PATIENT AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION.

A. Asahi, S. Okamoto, T. Mori, R. Watanabe, and Y. Ikeda. Keio BMT Program, Div. of Hematology, Keio University, School of Medicine, Tokyo, Japan

Chronic graft-versus-host disease (CGVHD) is a major complication following allogeneic stem cell transplantation. The incidence and clinical characteristics of CGVHD in transplant recipients receiving contemporary immunosuppressants are not well described in detail. To address this question, we prospectively investigated the clinical characteristics of de novo and quiescent CGVHD in Japanese transplant recipients.

Between January 1990 and December 1997, 113 patients with various hematologic disorders underwent allogeneic boe marrow transplantation at the Keio BMT Program. Among them 90 surviving 70 days or more posttransplant with sustained engraftment were enrolled into this study. All patients received T-cell non-depleted marrow from either HLA-matched sibling or unrelated donors. GVHD prophylaxis was attempted with cyclosporine or tacrolimus with short-term methotrexate. Immunosuppressants were tapered around day 50 and discontinued around day 180. The diagnosis of CGVHD was based upon both clinical and histological findings characteristic of CGVHD and exclusion of other causes.

The incidence of de novo and quiescent CGVHD was 51% (46/90) (14.4% de novo, and 36.7% quiescent). The median day of diagnosis of CGVHD was 155 days posttransplant ranging from 54 to 900 days. Mouth was most frequently affected (67.4%) followed by eye (55.8%), liver (51.2%), lungs (34.9%) and skin (32.6%). GI tract was affected in only one patient. Although two or more organs were affected in most cases, CGVHD in a single organ was also observed in 7-14% of cases (skin 7.1%, mouth 10.3%, lung 13.4%, eye 12.5%, liver 13.6%). Higher age of the patient, preceding acute GVHD, and the type of graft (sibling vs. unrelated donor) did not associate with higher incidence of CGVHD. With respect to the treatment, a complete response was observed most frequently in mouth GVHD (72.4%) followed by liver (68.2%), lungs (53.3%), skin (57.1%) and eyes (20.8%). The overall survival and event-free survival did not differ significantly in patients with or without de novo and quiescent CGVHD. However, de novo and quiescent CGVHD had significantly affected the quality of life within one year after transplantation. Our observations on CGVHD somewhat differ from previous reports in the western countries, and these differences may be attributable to differing genetic backgrounds between Japanese and western populations.

5

OUTPATIENT AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOLLOWING SHORT COURSE VAD IN MULTIPLE MYELOMA(MM).

M.R. Trus, L. Bordeleau, C. Bredeson, I. Bence-Bruckler, S. McDiarmid, L. Huebsch, H. Atkins, Ottawa Hospital Blood and Marrow Transplant Programme, Ottawa

We hypothesize that myeloablative therapy is more important for control of multiple myeloma than conventional chemotherapy and that equivalent disease control can be achieved with less chemotherapy if followed by transplantation. Also the transplantation can be performed safely in the outpatient setting.

A retrospective review of consecutive patients (pts) with at least Stage II multiple myeloma referred for autoHSCT was undertaken. A total of 42 pts were referred for autoHSCT, 6 pts were precluded because of co-morbid illnesses. The pts received a median of 3 cycles of VAD chemotherapy. Six pts did not respond to VAD. All treatments were performed as outpatients with patients admitted to hospital only for medical complications.

Pts required a median of 2 PBSC collections. One death from sepsis occurred during cyclophosphamide induced pancytopenia. A total of 35 pts underwent autoHSCT between March 1994 and January 1998. Most (91.4%) received myeloablative therapy consisting of melphalan 140 mg/m^2, etoposide 60 mg/kg and 1x 500cGy total body irradiation. Median time to engraftment for neutrophils was 10 days and platelets was 11 days. A median of 9 days was spent in the outpatient transplant unit and 17 days as inpatients. Nine pts (26%) suffered Grade 2-4 Bearman regimen related toxicity (RRT) and there were 2 deaths from RRT. CR was obtained in 12 pts (36%), a VGPR in 8 pts (24%), a PR in 12 pts (36%) and 1 patient had stable disease (3%). Seven pts relapsed (21%) and 2 pts (6%) have died during the follow up period. The median follow up from diagnosis was 75.8 weeks and 43.3 weeks from transplant. The relapse free survival from diagnosis was 73% at 2 years and 85% at 40 weeks post transplant. The overall survival from diagnosis was 90% at 2 years and 94% at 40 weeks post transplant.

The response rates are similar to other groups' experiences. This protocol is as effective as a larger number of cycles of standard dose chemotherapy followed by autoHSCT in the treatment of MM.

6

REMOBILIZATION OF PATIENTS WITH INADEQUATE INITIAL PROGENITOR CELL COLLECTIONS FOR AUTOLOGOUS TRANSPLANTATION.

A. Bashey, S. Corringham, P. Holman, E. Carrier, D. Ward, A.D. Ho, T.A. Lane, E.D. Ball and P. Law. Blood and Marrow Transplantation Program, University of California, San Diego, La Jolla, CA

A majority of patients undergoing autologous peripheral blood progenitor cell (PBPC) transplantation achieve target CD34$^+$ cell dose in a single series of mobilization and leukaphereses (LP). We analyzed 21 consecutive patients that failed to provide an adequate collection (>3 x 10^6 CD34 cells/kg) who then underwent a second mobilization a median of 15 days later (range 12-196 days). Initial mobilization consisted of G-CSF alone (10 μg/kg/day) (n=10), chemotherapy + G-CSF (8), chemotherapy + GM-CSF (2). Regimens for second mobilization were G-CSF + GM-CSF (10 μg/kg/d each) (12), chemotherapy + G-CSF (3), G-CSF only (20 μg/kg/d) (3). Median numbers of LPs performed with the first and second mobilizations were the same (median = 4, range 2-5). Fifteen patients achieved the target CD34$^+$ cell dose after the second collection. In 6 patients, the total collected CD34$^+$ cells were insufficient for autologous transplantation. Mean CD34$^+$ cells/kg/LP collection was higher for the second mobilization procedure (2.85x10^5/kg vs. 5.52x10^5/kg, p=0.015) as was the total WBC/kg/LP collection (1.98x10^8/kg vs. 3.40x10^6/kg, p=0.004). For the 12 patients undergoing second mobilization with the G-CSF+GM-CSF combination, the CD34$^+$ cell/kg/LP collection (4.94x10^5 vs. 2.53x10^5, p=0.083) and the total WBC/kg/LP collection (3.88x10^8 vs. 2.17x10^8, p=0.014) were greater than those achieved in the first mobilization (G-CSF alone 7, chemotherapy + G-CSF 5). A second mobilization procedure is effective in facilitating adequate PBPC collection in the majority of patients who fail to provide an adequate collection with the first mobilization. In this patient population, second mobilization using a G-CSF + GM-CSF combination appears to produce a larger progenitor cell yield than the initial mobilizing regimen. This effect may be related to the increased total dose of growth factor or a synergy between the growth factors. Agents with known synergy to G-CSF, e.g., stem cell factor should be evaluated in this context.

7

PROLONGED ERYTHROID APLASIA AFTER MAJOR ABO-MISMATCHED TRANSPLANTATION FOR CHRONIC MYELOGENOUS LEUKEMIA.

Jean M. Connors, Siobhan Mc Gurk, W. Hallowell Churchill, Joseph H. Antin, Richard J. Benjamin. Dana Farber /Partners Cancer Care and Brigham and Women's Hospital, Harvard Medical School, Boston, MA

The effect of graft ABO-blood group mismatch on erythroid engraftment after allogeneic bone marrow transplant (BMT) for chronic myelogenous leukemia (CML) is investigated.

Transfusion requirements and engraftment parameters were reviewed for 112 patients with stable (110) or accelerated (2) phase CML receiving related (60) or matched-unrelated (52), non T-cell depleted BMT over a 3.5 year period.

Twenty-two of 76 evaluable patients were transplanted over an ABO major-mismatch compatibility barrier. These patients showed a significantly increased delay in erythroid engraftment and in time to RBC transfusion independence when compared with ABO-identical or minor-mismatched recipients. No difference in granulocyte or platelet engraftment was evident. Erythroid engraftment usually occurred spontaneously without specific intervention. One patient was found to have erythroid hypoplasia at day 201 after BMT despite therapy with intravenous immunoglobulin and high dose erythropoietin. An anti-A titer of 16,000 was documented. This patient was successfully treated with an aggressive course of 18 plasmapheresis procedures with replacement with donor-type plasma.

Delayed erythroid engraftment is common after non T-cell depleted ABO major mismatched BMT in CML but rarely requires intervention other than transfusion support. Rare cases of refractory erythroid aplasia may be treated without additional immunosuppression by means of aggressive plasma exchange with donor-type plasma.

8

RANDOMISED TRIAL OF BLOOD CELL (BC) VERSUS BONE MARROW ALLOGENEIC TRANSPLANTATION: A STUDY FROM THE SOCIÉTÉ FRANCAISE DE GREFFE DE MOELLE OSSEUSE.

D Blaise, M Kuentz, C Fortanier, JH Bourhis, N Milpied, JP Jouet, N Fégueux, JM Boiron, P Bordigoni, L Sutton, M Attal, MP Schuller, F Guilhot, M Legros, JY Cahn, JP Moatti and M Michallet for the SFGM, Institut Paoli-Calmettes, Marseilles, France

We report a prospective multicentre randomised trial to compare allo BCT or allo BMT. Pts were randomised in 2 groups: in the BCT group, donor were collected by cytapheresis after priming with glycosaled (Lenograstim kindly provided by Laboratoire Rhône Poulenc Rorer, Montrouge) GCSF; in the BMT group, BM was aspirated under general anaesthesia. Pts did not receive G-CSF after transplant. Standard procedures for transplantation were used. 51 pts (37 ± 4; M/F = 26/25) with early leukaemia (AML=24; ALL=10; CML=17) are analysable (BCT=27; BMT=24). All donors (age: 40 (20-60); M/F=27/24) were HLA A,B,DR matched siblings. Groups were balanced for patient, donor and transplant characteristics. BC harvest led to the collection of a higher number of CD34$^+$ and CD3$^+$ cells than BM harvest (p=0.004 and p<0.001). Time to reach 0.5 and 1 x 10^9/l neutrophils was 5 and 3 days shorter in BCT group (p=0.007 and p=0.004). Pts in the BCT group reached 25 and 50 x 10^9/l platelets 10 and 18 days earlier (p=0.005 and p<0.001). This led to fewer platelet transfusions during the 180 first days post transplant (4 transfusions vs. 12 transfusions: p=0.001). This quicker haematological recovery was associated with a shorter hospitalisation duration. AGVHD was not increased nor CGVHD with this follow-up. Fifteen month probabilities of relapse and survival are not different. In addition, prospective and comparative economic evaluation of the first 6 months after transplant was performed. This haematological benefit was associated with a 20% decrease in the cost of the procedure over the first 6 months. Allo BCT leads to a dramatic improvement of both platelet and neutrophil reconstitution with absence short-term adverse effects for donors or any impairment to patient outcome.

9

OUTPATIENT MANAGEMENT AFTER BONE MARROW TRANSPLANTATION.

Melo. T.S.; Neves, M.L.; Pinheiro, C.T.; Torres, R.C.; Bouzas, L.F.; Tavares, R.C.; Tabak, D.G. Bone Marrow Transplantation Unit (CEMO), Instituto Nacional de Cancer, Rio de Janeiro, RJ, Brazil

Technological advances resulted in an increasing number of patients submitted to bone marrow transplantation (BMT) with a favorable outcome. The existance of adequate facilities and personnel can contribute to the reduction of complications. Here we compare distinct periods of outpatient follow up in our Unit after the establishment of a complete ambulatory care department. Patients (pts) were evaluated in 3 different periods: 25 pts from 4/93 - 3/94 (1st); 36 pts from 4/95 - 3/96 (2nd) and 60 pts from 4/97 - 3/98 (3rd). Initially, the outpatient clinic included only offices. On the 2nd period, an enlarged facility was created and on the 3rd period new personnel was trained and special care was delivered to pts and family members. In the 1st period, hospital discharge occurred a median of 30d after BMT (14-99), in the 2nd period 24d (10-71) and 22d (10-85) on the 3rd period. Only 4% of the pts were discharged prior to day 20 on the 1st period while 22.2% and 36.7% left the hospital prior to day 20 respectively on the 2nd and 3rd periods. Readmissions were more frequent on the 1st and 2nd periods when compared to the 3rd period (80% x 61% x 36%, respectively). The major cause of readmissions were infections (25% x 50% x 22.7%) and metabolic/gastrointestinal disturbances (30% x 36.5% x 31.8%), respectively in 1st, 2nd, 3rd periods. Prior to transplantation only 16.7% of the pts were admitted on the 3rd period when compared to 56% and 47.2% respectively in the 1st and 2nd periods. We have demonstrated our ability to improve our hospital stay through adequate nursing care in a special outpatient facility for patients submitted to BMT. Our analysis of cost reduction is underway.

10

DMSO IS NOT TOXIC TO HEMATOPOIETIC STEM CELLS.

R. Buckstein, K. Imrie, D. Spaner, M. Reis, P.Pinkerton*, I. Dube, A. Coovadia*, JB. Robinson*, S. Nanji*, P. Richardson*, A. Lima*, K. Hewitt* and NL. Berinstein. Toronto Sunnybrook Regional Cancer Centre. Toronto, Ontario*

Purpose: We evaluated the role of 'slow' stem cell infusions in mitigating the adverse side effects often attributed to the DMSO during 'bolus' injections. We determined the safety, efficacy and toxicity of this approach by measuring colony count formation, CD34+ counts, and viability of samples extracted from the first bag of stem cell graft. We also assessed patient symptoms during extended stem cell infusion times and the times to neutrophil engraftment and platelet transfusion independence. **Methods:** 5 consecutive patients who received high dose CBV chemotherapy followed by autologous stem cell transplant for relapsed follicular lymphoma were evaluated. Stem cells were collected via large volume leukapharesis and cryopreserved 1:1 in 20% DMSO, 10% plasma and 70% plasmalyte at -152°C. On the day of transplant, patients were premedicated with Solucortef, Benadryl, Ondansetron and Ativan. Stem cell bags were consecutively thawed, diluted with ACDA 20% of total volume and infused by a nurse. Three samples of 2 ml each were withdrawn from the first bag of thawed stem cells and at room temperature exposure times of 0,30 and 60 mins, the samples were washed once, resuspended and cells counted. Viability was measured by trypan blue exclusion. CD34% enumeration was by flow cytometry using ISHAGE guidelines. Cells were plated in triplicate at standard concentration in semisolid methylcellulose pre-tested anchorage independent culture dishes, and incubated for 10-14 days at 37°C in a 5% CO_2 environment. Progenitor cell enumeration was by standard criteria. **Results:** The median number of bags infused per patient was 3, with a median volume of 170 ml, a median total infusion time of 72 minutes and a median infusion rate of 2.67 ml/minute. Patients were largely asymptomatic during infusion.

Time	0	30	60	p
CFU-GM	41	56	34	NS
BFU-E	46	75	57	NS
CD34%	.23	.27	.21	NS
Viability%	71	73	75	NS

Median time to neutrophil engraftment was 11 days and 10 days to platelet independence.

Conclusion: A slower infusion of thawed stem cells is well tolerated, easily administered by nursing personnel and does not compromise engraftment rates, CD34% and progenitor cell counts or cell viability. Our study supports the routine use of slow infusions in stem cell autografting.

11

AUTOLOGOUS STEM CELLS TRANSPLANT (ASCTX) AFTER ASSISTED MECHANICAL VENTILATION (AMV) FOR ADULT RESPIRATORY DISTRESS SYNDROME (ARDS).

Canepa C., Orlando S., Saba S., Marino A., Nascimbene G., Godoy G., Mykietiuk A.BMT Unit, CUCAIBA, La Plata, Argentina.

Purpose.. To evaluate patients response to ASCTx that previously required AMV for ARDS.

Materials And Methods.. Retrospective analysis of clinical records and files of oncohaematology patients to establish the number of patients who underwent ASCTx that previously required AMV for more than 24 hs due to ARDS. The AMV was carried out at the BMT unit of C.U.C.A.I.B.A., La Plata, Argentina, with our medical staff under strict isolation.

Results.. During the period 6/1994 to 10/1998, 92 patients were subject to ASCTx; only two of them required AMV due to ARDS. Both patients suffered from A.M.L. and required AMV as a consequence of neutrophenic episodes, fever, and sepsis, with blood cultures positive to Streptococus viridans. Both patients recovered from their ARDS and later underwent ASCTx. At the time of update (98/10/31) both patients showed complete remission (CR) at +610 and +836 days, respectively.

Comments. AMV allowed these patients to be subject to ASCTx, be disease-free and in continous CR after +610 and +836 days. AMV did not affect later ASCTx.

12

SAFETY OF AUTOLOGOUS STEM CELLS TRANSFECTED WITH REVM10 INFUSED INTO HIV+ PATIENTS WITH CD4+ CELL COUNTS < 500 AFTER CONDITIONING WITH CYCLOPHOSPHAMIDE.

M.Carabasi, S.Deresinski, P.Bucy, A.Tilden, G.Shaw, S.Jackson, C.Kemper, T. Martin, and K.Squires

University of Alabama, Birmingham, Birmingham, AL, AIDS Community Research Consortium, Redwood City, CA, and SyStemix, Palo Alto, CA.

We have conducted a phase I study using autologous stem cells transfected with RevM10, a transdominant mutant of the HIV gene Rev that confers resistance to HIV. Transduced stem cells were infused into 24 HIV+ patients (pts) with CD4+ counts < 500. All pts were age 18 yrs or older, were on no or stable anti-retroviral therapy for 30 days prior to enrollment, had suitable I.V. access, and demonstrated adequate marrow and other organ function prior to treatment. Pts with concurrent malignancy, serious opportunistic infection, or receiving other immunomodulating agents or anti-retrovirals not covered by IND were excluded. Pheresis products were collected via peripheral venous access in a single session after mobilization with G-CSF. 11 pts (5 with CD4+ counts between 100 and 500, 6 with CD4+ counts < 100) were treated with transduced stem cells alone. 13 pts with CD4+ counts between 100 and 500 received cyclophosphamide (Cy) (1.2g/m^2 - 5pts, 1.8g/m^2 - 5pts, and 2.4g/m^2 - 3pts) prior to transduced stem cell infusion. All pts experienced fevers and myalgias due to G-CSF during mobilization. 9 pts, including 6 in the two highest dose Cy cohorts, became neutropenic after treatment with Cy, and 2 pts developed febrile neutropenia. All pts recovered uneventfully. Viral burden has been monitored in 19 pts for at least 3 mos., with 18/19 followed for 6 mos. No significant impact on viral burden has been detected, although no data are yet available on pts receiving the highest CY dose. In addition, no change has been seen in the absolute CD4+ cell count in any group, including 3 pts treated with the highest dose of Cy. Analysis of T and B cell function is ongoing. To date, transient low-level marking has been seen in PBMC assayed post-infusion in 2 of 3 pts treated with the highest dose of Cy. We conclude that autologous stem cells transfected with RevM10 can be safely used alone in HIV+ pts with very low (<100) CD4+ counts, or in combination with CY in pts with CD4+ counts between 100 and 500. Whether higher levels of marking can be obtained with more aggressive conditioning or different gene constructs is being investigated.

13

CORD BLOOD TRANSPLANTATION FOR PAEDIATRIC HAEMATOLOGLCAL DLSORDERS : THE HONG KONG EXPERIENCE

GCF Chan1, SY Ha1, TL Lee1, MHY Tang2, RHS Liang3, YL Lau1.Departments of Paediatrics1, Obstetrics2 & Medicine3, Queen Mary Hospital, The University of Hong Kong, Hong Kong, China

Objectives : To review our early experience of using cord blood transplantation (CBT) for the treatment of various types of childhood haematological disorders.

Methods : From Dec. 94 to Nov. 98 a total of 5 cases of CBT was performed in our hospital. Four patients had transfusion dependent thalassaemia (Thal)((thalassaemia major n=2, haemoglobin Bart disease n=1, (thalassaemia/haemoglobin E n=1) and one patient had acute T-lymphoblastic leukaemia (ALL) in second remission. The conditioning regimen was Bu20/Cyc200/ATG90 for the Thal (all Thal patients were less than 3 years old) and VP16(50)/Cyc100/TBI(12) for the ALL patient. The graft vs host (GVHD) prophylaxis was cyclosporin A & methotrexate. **Results:** The 4 Thal patients received sibling HLA-matched CBT (one case of 1-Ag mismatched) and the leukaemic patient received HLA-matched unrelated donor (MUD) CBT. T-cell depletion was not performed. The median age of the recipients was 2.8 yrs (range: 2.2 to 10 yrs). The median cord blood volume was 64ml (range 30-81ml). The median MNC was 5 (range 0.87-11.4)x10^7/Kg & the median CFU-GM was 2.2 (range 1.01-7.6)x10^4/Kg. All patients engrafted (neutrophil > 0.5x10^9/L for 2 consecutive days) and the median duration of engraftment was 24 days (range : 16 to 27 days). Four of them received G-CSF augmentation prior to engraftment. The median engraftment time for platelet (Plt>50x10^9/L) was 38 days (33-56 days). Three patients developed acute GVHD (grade 2 to 3) but mainly affecting the skin and responded to short course of pulse steroid treatment very well. The chimeric study by serial karyotyping on the 3 sex mismatched cases showed 100% donor cells after transplant. All Thal patients became transfusion independent and their median follow-up time was 15.5 months (range: 4 mos to 4 yrs). The leukaemic patient showed no sign of relapse but the follow-up time is still short. **Conclusions:** CBT is a feasible and reliable alternative for bone marrow transplantation. There is no increase in rejection noted in thalassaemia patients who underwent CBT. Similar to others finding, GVHD seems to be less severe and extensive in CBT as shown in our I-Ag mismatched and MUD cases.

14

ABX-CBL FOR THE TREATMENT OF STEROID REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD).

H.J. Deeg, B. Bolwell, G. Long, F. Schuening, R. Rifkin, J. Cunningham, B. Blazar, T. Furlong, N. Havrilla, J. Lipani. Fred Hutchinson Cancer Research Center, Seattle, WA; The Cleveland Clinic, Cleveland, OH; Duke University, Durham, NC; University of Wisconsin, Madison, WI; Rocky Mountain Cancer Center, Denver, CO; University of Minnesota, Minneapolis, MN; and Abgenix, Inc., Fremont, CA

Allogeneic marrow ansplant recipients who develop acute GVHD despite GVHD prophylaxis generally receive first-line therapy with glucocorticoids. However, with this "standard" treatment, complete responses are achieved in only 20-25% of patients, thus, the majority of patients require additional therapy. Monoclonal antibody (MAB) ABX-CBL is a murine IgM, reactive with activated T, B and NK cells, and recently shown to be directed at CD147. In pilot studies, this MAB had activity in the treatment of rejection of renal allografts and in children with steroid-refractory GVHD. The present dose escalation trial started at 0.01 mg of ABX-CBL/kg, escalating over 0.1 to 0.3 to 1.0 mg/kg given daily for 7 days and for four additional doses over the ensuing 2 weeks. At seven transplant centers, 26 patients have been enrolled. Patient age ranges from 1 to 46 (median 35) years; 17 patients were male and nine female. The underlying diagnoses included acute or chronic leukemia, lymphoma and myelodysplasia. The donor was related (HLA-identical or one antigen mismatched) in 13 and unrelated in 13 cases. The extent of GVHD at the time of enrollment included skin involvement only in five, liver only in one, and various combinations of skin, intestinal and hepatic involvement in the remaining patients. Eight patients given 0.01 mg/kg (anticipated to be non-effective), and six patients given doses of 0.1 mg/kg experienced no side effects. Of the subsequent six patients given 0.3 mg/kg, three developed severe muscle cramping which was considered dose-limiting toxicity. Thus the dose was de-escalated to 0.2 mg/kg and six patients were treated at that level. Overall, 15 of 26 patients (13 of 18 given (0.1 mg/kg) had clinical responses, including two partial responses at 0.01 mg/kg. In six patients, responses were complete and no additional therapy was required. Four patients who had responded had a flare of GVHD and were retreated with ABX-CBL at 0.3 mg/kg/day. Two had a response; in two, treatment was discontinued because of severe muscle pain. In conclusion, ABX-CBL offers effective therapy of acute GVHD in steroid refractory patients. The maximum tolerated dose of antibody ABX-CBL has been identified. This MAB warrants further investigation in allogeneic marrow transplant recipients.

15

PHASE I STUDY OF HIGH-DOSE TOPOTECAN AND ALKYLATING AGENTS FOR ADVANCED OVARIAN CANCER.

M. Donato D. Gershenson, J.T. Wharton, A. Aleman, C. Ippoliti, D. Bodurka, C. Levenback, M. Bevers, J. Wolf, R. Freedman, J. Gajweski, R. Bast and R.E. Champlin. M.D. Anderson, Cancer Center, Houston TX.

Topotecan (TOPO) is a promising agent for platinum refractory ovarian cancer (CA) but its use in combination is limited by myelosuppression. The purpose of this study was to establish the maximum tolerated dose (MTD) of TOPO when used with alkylating agents followed by peripheral blood stem cells (PBSC). 22 patients (pts) with advanced ovarian CA received a total of 26 cycles of TOPO with cyclophosphamide (CY) and melphalan (MEL) followed by PBSC. Pts received TOPO over 30 min daily on days(d) -6 to -2; CY 1 g/m^2/d d -6, -5, -4 and MEL 70 mg/m^2/d d -3, -2. PBSC were infused on d 0 with G-CSF. Median age was 45(21-61). Disease status pre-transplant: chemoresistant with PD(6 pts), relapsed disease in PR(7 pts), positive 2nd look(7 pts), (2nd CR(2 pts). 4pts had (1 prior carboplatin-based transplant. Toxicity using the Bearman scale, was limited to grade 2 mucositis and diarrhea in a total of 6 and 2 pts respectively, at dose levels 1, 4, 6. Engraftment kinetics did not vary with dose levels. Median time to neutrophils (0.5x10^9/(L was 9 days (8-11) and platelets (50 x 10^9/(L: 14days (7-24).

DOSE LEVEL	1	2	3	4	5	6	7
TOPO mg/m²/d	**1.25**	**1.5**	**1.75**	**2.0**	**2.25**		
2.5	**2.75**						
Number of pts.	**2**	**3**	**3**	**3**	**5**	**9**	**1**

55% of the cycles were associated with neutropenic fever. Disease status post transplant: 76%CR, 19%PR, 5%SD. Overall response rate for patients with measurable disease which includes all the chemoresistant pts. was 91.7%. In conclusion, the TOPO, CY and MEL combination is a promising new regimen, feasible in heavily pretreated pts. Non-hematological toxicity of TOPO was minimal and the MTD has not yet been established. The regimen was very active and a phase II study should follow.

16

CONTROL OF ASPERGILLUS PNEUMONIA WITH REPEATED DONATION OF G-CSF ELICITED GRANULOCYTES.

Reggie E. Duerst, CN Abboud, and J Liesved, Univ Rochester, Rochester NY

Fungal infection, (esp. aspergillus), is often a contraindication to high-dose therapy and hematopoietic stem cell rescue (HSCR). The prolonged period of neutropenia results in great risk for progression of infection. Granulocyte transfusions (Gran Tran) have been used as a prophylactic measure or as a therapeutic agent for patients with proven infection and neutropenia. We report the use of directed GranTran, from father to ABO compatible son (5 yo), following HSCR for refractory AML. Aspergillus infection was con101 firmed 3 weeks prior to HSCR by broncho-alveolar lavage (BAL). Unrelated umbilical cord blood matched at 4 of 6 HLA antigens was used as the source of stem cells. G-CSF was used to elicit neutrophilia in the donor, and an apheresis product with high cell content.

Cytoreduction was comprised of fractionated total body irradiation, melphalan and anti-thymocyte globulin. GVHD prophylaxis was comprised of cyclosporin A and corticosteroids. Beginning on d 0, the father was treated with G-CSF (480 mcg). Apheresis followed 12 hr later. The recipient received G-CSF daily. The cell products were divided into two parts. One portion was irradiated and infused within 6-8 hr of collection (days 1, 4, 7, 10, 14 and 17), the other was stored at 4 (C overnight, then irradiated and infused the following day. Apheresis products contained from 4.6 to 10.4 x10^{10} cells. Cell doses for the recipient (17 kg) ranged from 1.35 to 3 x10^9 cells/kg. After initiation of the Gran Tran, the patient's WBC was < 100/ul only once (day +7). Because of mucositis, itraconazole was replaced by liposomal amphotericin-B (lipAmB) days 12-25. G-CSF was discontinued on day +28. CT scan on day +28 revealed clearing of the infiltrates noted pre-HSCR. Corticosteroids were tapered off. CT scan day +77 showed redevelopment of infiltrates in the previously noted locations. Open biopsy demonstrated aspergillus by cytology and lipAmB has been restarted.

Anti-fungal therapy and GranTran during the immediate post-HSCR period led to improvement, but not eradication of the aspergillus pneumonia. A pilot study to evaluate the feasibility of directed GranTran for patients at high-risk for infectious complications will be pursued.

17

A SINGLE ADMINISTRATION OF R-METHUG-CSF-SD/01 (SD/01) SIGNIFICANTLY IMPROVES NEUTROPHIL RECOVERY FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION.

A.M. Farese, L. Roskos, E. Cheung*, R.B. Stead, S.M. Yin*, T.J.MacVittie. Greenebaum Cancer Center, Baltimore, MD, and Amgen Inc, Thousand Oaks, CA*

Conventional daily administration of Filgrastim is effective in reducing the duration of severe neutropenia following myelosuppressive cytotoxic therapy or myeloablation and stem cell transplantation. SD/01, a PEGylated form of Filgrastim (r-metHuG-CSF), demonstrated enhanced biological and pharmacokinetic (PK properties in normal rhesus monkeys (n=3). Following a single injection of SD/01 at 300 (g/kg, white blood cells (WBC) and plasma SD/01 levels remained above baseline levels (BL) for 7 days. PK simulations indicated that effective plasma concentrations(2.5 ng/mL based on pharmacodynamic modeling) would be maintained for extended periods during neutropenia: therefore, dose levels of 100 and 300 (g/kg were selected for evaluation. We investigated the ability of SD/01 to modify neutrophil regeneration in a monkey model of bone marrow (BM)-derived mononuclear cell (MNC) transplantation. On d0, animals were myeloablated by total body exposure to 920 cGy, 250 kVp x-irradiation (TBI) and within 2 hours of exposure were infused with 1 x 10^8 AuBM-MNC/kg bw. The Au-BMT controls (n=9) received 0.1% autologous serum (AS). Following myeloablation and AuBMT, SD/01 (300 (g/kg, sc, n=4 or 100 (g/kg, sc, n=3) was administered on d1, or Filgrastim (10 (g/kg/d, sc, n=4) was administered on a daily basis until an absolute neutrophil count (ANC) of 3,000/(L was attained (range d12-17). Animals were clinically supported with antibiotics, fresh irradiated whole blood and fluids as needed. Bone marrow-derived clonogenic activity was evaluated prior to and on days 7, 14, 21 and 46 post TBI and AuBMT. Plasma samples for PK analysis were obtained through d11 post TBI, and complete blood counts were monitored for 50 days post TBI and AuBMT. Both SD/01 and Filgrastim significantly ($P < .05$)* improved neutrophil recovery.

TREATMENT	DURATION (DAYS) ANC < 500/µL	ANC NADIR (/µL)	REC TO BL (DAY)	ANTIBIOTICS REQUIRED(DAYS)
0.1% AS	**11.1**	**28**	**21**	**16.9**
SD/01 300 µg	**3.3***	**544**	**10**	**7.7**
SD/01 100 µg	**4.0***	**389***	**14**	**10.0**
Filgrastim	**6.5***	**148***	**13**	**9.3**

Neither SD/O1 or Filgrastim significantly improved platelet recovery. Effective plasma concentrations were maintained in myelosuppressed animals until after onset of hematopoietic recovery, consistent with self-regulating, ANC-dependent properties of SD/01 elimination. A single dose of SD/01 is at least as effective as daily Filgrastim and results in a significant improvement of neutrophil recovery following myeloablation and AuBMT in rhesus monkeys.

18

A NON-MYELOABLATIVE REGIMEN OF FLUDARABINE AND CYCLOPHOSPHAMIDE INDUCES MIXED CHIMERISM IN AN F1-INTO-PARENT MURINE MARROW REJECTION MODEL.

Matthew Petrus, Ronald E. Gress, and Daniel Fowler; Medical Oncology Branch, National Cancer Institute, Bethesda, MD

Clinical evidence suggests that fludarabine contributes to the prevention of marrow graft rejection in the setting of non-myeloablative allogeneic BMT. In light of this evidence, we developed an F1-into-parent murine marrow rejection model to evaluate factors that might optimize this transplant strategy. Host B6 mice were treated with high-dose total body irradiation (950 cGy), sublethal irradiation (600 cGy), or a combination of fludarabine (flu) and cyclophosphamide (cy) [100mg/kg/day and 50 mg/kg/day, respectively; 18-27 days of treatment]. Compared to recipients of 950 cGy irradiation (n=6), flu/cy recipients (n=6) had equivalent or greater levels of lymphocyte depletion just prior to the transplantation of T cell-depleted B6D2F1 marrow (flu/cy recipients versus TBI recipients: CD4#/spleen of 0.04M and 0.55M, p=.031; CD8#/spleen of 0.07M and 0.46M; p=.24; CD19#/spleen of 0.32M and 0.82M; p=.17). In marked contrast, the flu/cy recipients had much higher levels of Mac-1^+ myeloid cells/spleen than recipients of 950 cGy irradiation (4.6M versus 0.56M, p=.03); the level of Mac-1^+cells in the flu/cy recipients was not decreased relative to untreated B6 control mice (4.6M versus 2.9M/spleen; p=.14). Mice treated with 950 cGy irradiation displayed a high level of F1 chimerism as determined by two color flow cytometry at day 30 post-BMT (93% F1 chimerism; n=9). Mice given 600 cGy irradiation had very low level F1 chimerism (3.5(0.4%, n=8); in contrast, mice treated with Flu/cy had higher levels of F1 chimerism (8.6 (2.2%, n=13, p=.04). Further follow-up is required to evaluate the long-term stability of the mixed chimerism observed in the flu/cy recipients. These data indicate that preparative regimens containing a combination of fludarabine and cyclophosphamide have potential for the prevention of marrow rejection across fully-MHC disparate barriers without myeloid ablation.

19

EX VIVO EXPANDED, RETROVLRALLY TRANSDUCED CYTOTOXIC T-LYMPHOCYTES (CTL) ENHANCE ENGRAFTMENT OF HAPLOIDENTICAL MARROW.

GE Georges, R Storb, JD Thompson, and RA Nash, Fred Hutchinson Cancer Research Center and University of Washington, Seattle, WA

Using the well-established dog model of allogeneic hematopoietic stem cell transplantation, we asked if retrovirally transduced donor derived, recipient-specific CTL can prevent rejection of histoincompatible marrow.

CTL were generated by bulk mixed lymphocyte culture between DLA-haploidentical littermates. To track CTL following in vivo infusion, we transduced CTL with the retroviral vector LGFPSN expressing green fluorescence protein (GFP) and *neo*^{R}. Following 4- week culture that included transduction, G418 selection and expansion, CTL had 60-80% specific lysis and >99% of CTL were $CD3^+$, >95% GFP^+ and >76% were $CD8^+$. A median CTL cell dose of $2.0x10^7$/kg recipient weight was infused into 7 DLA-haploidentical littermates following 9.2 Gy total body irradiation (TBI). The median unmodified marrow cell dose infused was $4.5x10^8$ total nucleated cells/kg or $3.9x10^6$ CD34+/kg. No post grafting immunosuppression was given.

Seven of 7 haploidentical recipients engrafted (ANC>500/(L, median day +8) and platelets (>50K); all 7 recipients developed multi-organ severe (grade IV) graft versus host disease (GVHD) confirmed by histopathology. Fluorescence cytometric analysis of recipient peripheral blood after transplant revealed a sharp increase in the absolute number of circulating GFP^+CTL ($1.0\text{-}1.5x10^4$/mL) on days +5 to +6, with a subsequent rapid fall in GFP^+ cells at the onset of engraftment. As controls, 8 dogs received DLA-haploidentical marrow only following 9.2 Gy TBI: 2 engrafted and developed GVHD, 6 had graft rejection.

These results show that ex vivo expanded, retrovirally transduced CTL enhance engraftment of DLA-haploidentical marrow in the setting where graft rejection is expected. Future studies will test if transduced CTL addback to T-cell depleted marrow can safely achieve engraftment without GVHD using an inducible suicide gene, herpes simplex virus thymidine kinase.

20

CONVERSION FROM MIXED TO ALL DONOR HEMATOPOIETIC CHIMERISM FOLLOWING INFUSION OF MINOR HISTOCOMPATIBILITY ANTIGEN-SPECIFIC DONOR LYMPHOCYTES IN DOGS.

GE Georges, R Storb, C Yu, B Torok-Storb, JD Thompson, B. Bruno and RA Nash, Fred Hutchinson Cancer Research Center and University of Washington, Seattle WA

Stable mixed hematopoietic chimerism can be established in dog leukocyte antigen (DLA)-identical littermates following sublethal total body irradiation (200 cGy TBI) before and immunosuppression with mycophenolate mofetil and cyclosporine (CSP) after stem cell transplantation. We asked if donor lymphocyte infusions (DLI) could be used to convert mixed to all donor chimerism.

Eight dogs with stable mixed chimerism were given unmodified DLI between day 36 to 414 with an infused cell dose of $1.5x10^7\text{-}2.0x10^8$ $CD3^+$cells/kg. Three received a second unmodified DLI on day 213 to 286 with $1.0x10^8\text{-}2.6x10^8$ $CD3^+$/kg. Chimerism status was assessed by quantitative PCR phosphorimage analysis using informative microsatellite markers with peripheral blood DNA, for 4-8 months after each DLI. There was no significant change in percent donor engraftment following each unmodified DLI in these 8 mixed chimeras.

DLI donors were then sensitized to recipient minor histo-compatibility antigens (mHA) in vivo with weekly subcutaneous injections of recipient skin grafts x 4. Eight mixed chimeras received mHA-specific DLI on day 201 to 651 at a cell dose of $2.0x10^7\text{-}9.3x10^7$ $CD3^+$/kg. Within 4 to 10 weeks after DLI, all 8 recipients converted to >95% donor chimerism in both lymphoid and myeloid cells. Two recipients developed grade II skin graft versus host disease (GVHD) 3 to 4 weeks after DLI that responded to CSP treatment. One recipient developed severe marrow aplasia without GVHD. The remaining 5 recipients had no complications from mHA-specific DLI.

These results show that a non-myeloablative transplant regimen to establish mixed hematopoietic chimerism provides a platform for adoptive immunotherapy that has clinical potential to treat patients with malignant diseases. Conversion to all donor hematopoiesis requires infusion of donor lymphocytes specific for host mHA.

21

HYDROXYCHLOROQUINE FOR THE TREATMENT OF CHRONIC GRAFT-VERSUS-HOST DISEASE

Andrew Gilman, Ka-Wah Chan, Mark Mogul, Christopher Morris, Fred Goldman, Michael Boyer, Emanuel Cirenza, Amitabha Mazumder, Richard Cahill, Stanley Frankel, and Kirk Schultz.Children's Mercy Hospital, Kansas City, MO, University of Texas/MD Anderson Cancer Center, Emory University, Atlanta, GA, University of Cincinnati, Cincinnati, OH, University of Iowa, Iowa City, IA, Albany Medical College, Albany, NY, Georgetown University, Washington, DC, and University of British Columbia, Vancouver, BC

A Phase II trial of hydroxychloroquine (HCQ) for chronic graft-versus-host disease (GVHD) was conducted. HCQ is a 4-aminoquinoline antimalarial used for the treatment of autoimmune diseases. HCQ interferes with antigen processing and presentation, cytokine production, and cytotoxicity. Thirty-nine patients with steroid-resistant or steroid-dependent GVHD or who were experiencing toxicity from medications needed to control their GVHD were enrolled from 8/94 to 11/98. Patients were treated with HCQ 12 mg/kg/day (adult dose 800 mg/d) for up to 1 year after achieving target HCQ levels. Twenty-nine patients (17 children, 12 adults) are evaluable (8 not evaluable because of an inadequate therapeutic trial, 2 too early to evaluate). Donor type was matched family member (16), matched unrelated donor (9), and haploidentical family member (4). Onset of chronic GVHD was progressive (12), quiescent (14), and *de novo* (3). Two complete and 13 partial clinical responses were seen for a response rate of 52% (15/29). All responders also had a >50% decrease in their dose of steroids. Improvement was most common in skin, oral, and hepatic involvement. Thrombocytopenia also often improved. There was no significant difference in the response rate based on the patient age, type of donor, type of onset of GVHD, or platelet count at study entry. Thirty-eight patients are evaluable for toxicity (one lost to follow-up). HCQ can cause visual scotomata, but these rarely occur before two years of therapy. No retinal toxicity was seen. The most common side effects were gastrointestinal symptoms, which occurred in three patients (8%). HCQ has significant clinical activity for chronic GHVD. The Children's Cancer Group is beginning a randomized, placebo-controlled, double-blinded Phase III trial of HCQ.

22

EXPERIENCE WITH PERIPHERAL BLOOD STEM CELL (PBSC) TRANSPLANTATION IN A CENTER OF MEXICO

Gómez-Morales E, Sánchez-Valle E, Campos G, Pedraza M, Chávez G, Pizzuto-Chávez J. Hospital de Especialidades, Centro Médico Nacional Siglo XXI, IMSS, México City, México.

The goal of this work is to show our experience in the use of PBSC in transplantation setting or their complications. Our protocol involved the mobilization with G-CSF at dose of 10 ug/Kg/day, subcutaneously for 5 days. PBSC were obtained with the special program of CS3000 machine. The volumen processed was 10 liters. Nine patients (pts) underwent PBSC infusion for transplantation, 7 of them in the autologous setting and the others in the allogeneic setting. Five pts had ANLL, 2 pts had ALL and the other two had MDS and CML respectively. Median age was 30, range 17 to 50 yrs; 5 female, 4 male. PBSC for autologous transplantation were harvested at 4°C without cryopreservation at long 5 days. Median MNC obtained was 6.3, range 3.8 to 13.0 x 10^8 MNC/Kg. Leucocyte engrafment occurred between days 14 to 24, median 21. Two pts had very slow platelet engrafment more than 100 days. Addicionally, 6 pts received PBSC for the treatment of relapse (2 ALL), graft failure (2 AA/ 1 ANLL) or Epstein Barr virus infection after BMT (1). The counts of MNC in this cases was similar to the others procedures. Two pts with relapse, who received continuous PBSC infusion developed pancytopenia and graft versus host disease G-IV. One pt with Epstein Barr virus infection and monoclonal gammopathy had a good response with control of infection. Two pts with AA had graft failure and they did not show response after PBSC infusion. One pt with ANLL received PBSC infusion for graft failure secondary to severe infection, she had not response. Conclusion: The approach with PBSC in transplantation was safe and the engrafment occurred in short time. Unfortunately, three patients developed platelet transfusion refractoriness. The use of PBSC for treatment of relapse or graft failure was unsuccessfully, because we observed severe pancytopenia and severe graft versus host disease. The use of PBSC for pts with Epstein Barr infection after BMT is a good option. It is necessary to obtain the minimal dose of CD34+ and CD3+ for achieve response in patients with relapse.

23

PHENOTYPIC ANALYSIS OF CD4 CELLS POST STEM CELL TRANSPLANT (SCT): PROLONGED PERSISTENCE OF AN ACTIVATED PHENOTYPE AND INCREASED PROPORTION OF TH 2 CELLS IN RECIPIENTS OF MATCHED UNRELATED DONOR (MUD) STEM CELL TRANSPLANTS (SCT).

M Grimley, D Marmer, S Lee, A Filipovich. Children's Hospital Medical Center, Cincinnati, OH, USA

Animal models have shown that the balance between Th 1 and Th 2 cells can affect the recovery of both cell-mediated and humoral immunity post SCT. We examined the expression of activation markers (HLA-DR and Ox40) and Th2 markers (CD30+ and/or CD7-) on CD3+CD4+ T cells at various time points post SCT using multiparametric FACS analysis on peripheral blood from SCT recipients. The results for matched sibling donor (MSD) and matched unrelated donors (MUD) are shown in the table. Patient results were significantly different ($p<0.05$) from normal controls except HLA-DR and Ox40 in MSD recipients at 6 mos post SCT.

CELL SURFACE MARKER	SCT TYPE	30 DAYS	100 DAYS	6 MONTHS	NORMAL CONTROLS
CD30+	MSD	1.8 +/- 1.6	1.2 +/- 0.6	0.5 +/- 0.5	0.35 +/- 0.33
	MUD	2.8 +/- 1.8	2.2 +/- 1.9*	2.2 +/- 1.8*	
CD7-	MSD	54 +/- 25	54 +/- 19	34 +/- 23	15.6 +/- 7.7
	MUD	51 +/- 10	49 +/- 18	43 +/- 24	
HLADR +	MSD	33 +/- 13	23 +/- 12	17 +/- 2	11.1 +/- 18.4
	MUD	31 +/- 18	45 +/- 22*	35 +/- 22*	
Ox40+	MSD	14 +/- 11	11 +/- 4.3	8.5 +/- 1.2	5.2 +/- 5.8
	MUD	20 +/- 13.2	23 +/- 12.7*	17 +/- 12.5*	

Data expressed as mean % expression of marker on CD4+ cells +/- 1 S.D. * = $p < 0.05$ comparing MUD to MSD at different time points

Recipients of MUD SCT demonstrate persistent skewing towards a Th2 phenotype as well as an activated CD4+ phenotype throughout the first 6 months post SCT. This Th2 skewing may inhibit the development of cell mediated immunity and contribute to the delayed immunoreconstitution in recipients of MUD SCT.

24

PROLONGED DEFICIENCY OF IN VITRO SYSTHESIS OF IL-12 AND TYPE 1 CYTOKINES IN RECIPIENTS OF ALLOGENEIC STEM CELL TRANSPLANT (SCT).

M Grimley, S Lee, A Marton, A Filipovich. Children's Hospital Medical Center, Cincinnati, OH, USA

Many of the late complications following mismatched stem cell transplant can be traced to the prolonged recovery of cell-mediated immunity. To understand the mechanisms potentially underlying this extended immunodefiency, we have begun to quantitate the generation of regulatory cytokines by mononuclear cells from transplant recipients collected at various time points post SCT and activated in vitro. PBMC were stimulated in culture with Staph Protein A (SAC) for IL-12 or Phtyohemagglutinin (PHA) for (-IFN, IL-4, IL-10 and IL-2. Cytokine production was quantified by ELISA of culture supernatants.

TIME DONOR	100 DAYS TYPE	6 MONTHS POST SCT	1 YEAR POST SCT	NORMAL SCT	CONTROLS
Mean γ-IFN	MSD	575	354	869	1761
	MUD	239	157	1052	
	Auto	2648	431	2326	
Mean IL-2	MSD	1390	1683	529	2391
	MUD	552	1013	367	
	Auto	1481	270	1636	
Mean IL-12	MSD	1.1	0.2	3.6	6
	MUD	1.1	1.1	0.8	
	Auto	0.3	*	11.0	

MSD=Matched Sibling donor; MUD=Matched Unrelated Donor; Auto=Autologous recipient. Mean IL-12, IL-2 and (-IFN production in pg/ml. * -no data available.

Our data reveal decreased IL-12 production in all SCT recipients initially, with normalization in MSD and Auto SCT patients by 12 months. In MUD SCT patients, IL-12 production remains depressed beyond 1 year post SCT. Overall, allogeneic recipients have low in vitro production of Type 1 cytokines compared to recipients of autologous SCT throughout the first posttransplant year.

25

TYPE 1 AND TYPE 2 T-CELLS IN THE STEM CELL GRAFT AND POST-TRANSPLANT VIRAL INFECTION.

TG Gross, JC Lynch, JR Davis, SJ Pirruccello, EC Reed, MR Bishop, ZS Pavletic, JO Armitage, A Kessinger, SR Tarantolo. University of Nebraska Medical Center, Omaha, NE. Children's Hospital Medical Center, Cincinnati, OH

Previously, we showed that an increase in type 1 ((-IFN+) T-cells, and a higher ratio of type 1 (T1) to type 2 (T2) (IL-4+) T-cells in the stem cell graft correlates with developing acute GvHD. We hypothesized that a lower T1/T2 ratio in the stem cell graft may pedict patients (pts) at risk for post-transplant viral complications. Fifteen pts received related, HLA-matched, G-CSF mobilized, allogeneic blood stem cell grafts. All pts received the same preparative regimen, GvHD prophylaxis, and CMV prophylaxis, monitoring and therapy. The content (% of cells) and dose (x10^9 cells/kg) of T1 and T2 cells in the stem cell graft were determined using intracellular cytokine staining and multiparametric FACS analysis. Twelve pts were evaluable for viral complications up to day +100. Four pts developed CMV infection, one pt died of CMV pneumonia. One pt developed EBV associated post-transplant lymphoproliferative disease, and 1 pt died of a disseminated adenoviral infection. Median values for each group are reported, p>0.05 was considered not significant (NS).

	%T1	DOSE T1	%T2	DOSE T2	T1/T2
CMV	**1.9**	**0.3**	**0.2**	**0.1**	**5.5**
No CMV	**0.5**	**0.2**	**0.8**	**0.4**	**1.0**
p=value	**NS**	**NS**	**NS**	**NS**	**0.03**
Viral Complication	**1.5**	**0.2**	**0.3**	**0.1**	**3.9**
No Viral Complication	**0.5**	**0.2**	**0.8**	**0.4**	**1.0**
p=value	**NS**	**NS**	**0.05**	**0.05**	**NS**

These results suggest that a relative increase in T2 cells and/or decrease in T1 cells in the stem cell graft may identify patients at lower risk of post-transplant viral complications.

26

EX VIVO EXPANSION OF CANINE DENDRITIC CELLS.

H. Hägglund, G. Mathioudakis, B. Bruno, G. Georges, J. Gass, P. McSweeney, R. Storb and R. Nash. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle

This is the first report of ex vivo expansion of canine dendritic cells.

Material and method: CD34+ and CD34- canine bone marrow cells (10^5/ml) were cultured in Iscoves medium with, GM-CSF and flt3-ligand from day 0 and TNF- (from day 7. The cytokine concentrations used were GM-CSF 5, 50 and 500 ng/ml, flt3-ligand 0.2 and 20 (g/ml, and, TNF-(10, 100 and 250 ng/ml. Cell count and flow cytometry (FACS) were done on day 9 and a mixed lymphocyte culture (MLC) was established to assess function.

Results: The cell number, "DC-like" phenotype or alloreactivity did not increase with increased GM-CSF, flt3 and TNF-(doses.

	CD34+ GM-CSF 5ng/ml	CD34+ GM-CSF 50ng/ml	CD34+ GM-CSF 500ng/ml	CD34- GM-CSF 5ng/ml	CD34- GM-CSF 50ng/ml	CD34- GM-CSF 500ng/ml
Cellcount (10^3/ml)	**360**	**350**	**150**	**70**	**50**	**40**
	(340-425)	**(300-410)**	**(100-290)**	**(40-90)**	**(30-60)**	**(20-60)**
Phenotype (%)						
MHC II+/CD14-	**44**	**n.d.**	**52**	**5**	**n.d.**	**2**
	(41-52)		**(44-59)**	**(4-7)**		**(2-2)**
CD1c+/Cd11c+	**48**	**n.d.**	**46**	**14**	**n.d.**	**18**
	(39-52)		**(43-49)**	**(2-21)**		**(14-20)**

MLC (CPM)	GM-CSF 5NG/ML	GM-CSF 50NG/ML	GM-CSF 500NG/ML
CD34+ - derived vs URD[2]	**6105**	**1703**	**6996**
CD34- - derived vs URD	**1972**	**919**	**1057**

All numbers are median (range). [1]The cultured cells were split on day 5. CD34+ -derived cells were irradiated cultured CD34+ cells (stimulators), CD34- -derived cells were irradiated cultured CD34-cells (stimulators). [2]URD; unrelated mononuclear donor cells (respondors), MLC; mixed lymphocyte culture, cpm; counts per minute, n.d.: not done

The cultured CD34+ cells: 1) had a 5 fold expansion in total cell number, 2) showed a morpholog consistent with a DC phenothype; MHC Class II+, CD1c+, CD11c+, CD34- and CD14-, 3) and showed increased allostimulatory activity compared to cultured CD34- cells.

Conclusion: GM 5ng/ml, flt3 0.2(g/ml and TNF-(10ng/ml is sufficient for expansion of "DC-like" cells from CD34+ cells. Future studies will determine if infusion of DC will influence engraftment, GVHD and tolerance in a dog transplant model.

27

TRANSPLANTATION OF PERIPHERAL BLOOD PROGENITOR CELLS FROM UNRELATED DONORS.

H.Hägglund, E.Sparrelid, M.Remberger, B.Lönnqvist, P.Ljungam, J.Aschan, G.Kumlien, P.Hentschke, S.Carlens, J.Mattsson, J.Winiarski, O.Ringdén. Allogeneic Stem Cell Transplant Unit and Depts. of Clinical Immunology, Infectious Diseases, Hematology, Pediatrics and The Blood Bank, Huddinge Hospital, Sweden

Purpose. Transplantations of peripheral blood progenitor cells (PBPC) from related donors are increasing rapidly. However, the experience using unrelated PBPC is limited. We here report single-center results of unrelated PBPCT compared with unrelated BMT.

Methods. Sixteen patients with hematological diseases received PBPC from unrelated donors. These patients were compared retrospectively with 16 recipients of unrelated BM and were matched for diagnosis, disease stage, age, conditioning and GVHD prophylaxis. All donors were HLA-A, -B and -DR compatible. The donors of PBPC were treated with G-CSF, 10 (g/kg/day, for 4-5 days and follow-up of the Swedish donors (n=11) are scheduled 3 months, 1 and 5 year after donation.

Results. The PBPC graft contained a significantly higher number of MNC, CD34+ and CD3+ cells (p<0.01). The median follow-up time was 16 (7-34) and 24 (7-41) months in the PBPC and BM groups, respectively. All 32 patients had neutrophil engraftment. Time to ANC>0.5x10^9/l was 6 days faster in the PBPC group compared to the BM group (p=0.003). The cumulative incidence of acute GVHD grades II-IV was 44% in the PBPC group and 32% in the BM group, respectively (ns). The cumulative incidence of grades III acute GVHD was 13% in both groups. No patient experienced acute GVHD grade IV. The cumulative incidence of chronic GVHD was 61% in both groups. The 1-year probability of TRM was 25% and 43% (ns), 2-years probability of relapse was 24% and 35% (ns), and 2-years probability of patient survival was 59% and 43% (ns), in the PBPC and BM groups, respectively. In none of the Swedish donors were severe side effects of the procedure reported.

Conclusion. This preliminary experience suggest that unrelated PBPC is a safe procedure both for the donor and patient.

28

LYMPHOCYTE RECOVERY AFTER ALLOGENEIC PERIPHERAL BLOOD TRANSPLANTATION (APBT) CORRELATES WITH THE SEVERITY OF REGIMEN RELATED ORGAN DYSFUNCTION (OD).

WD Haire, ZS Pavletic, SR Tarantolo, BG Gordon, MR Bishop. University of Nebraska Medical Center, Omaha, NE

Delayed lymphocyte recovery after APBT is associated with increased mortality (BMT 21:33, 1998). Neither the cause of this delayed recovery nor the mechanism of death is understood. Death in the early post-transplant period is generally due to the Multiple Organ Dysfunction Syndrome (MODS), a syndrome felt to be caused by abnormal immune and inflammatory responses to the preparative regimen and other, less well defined parameters (JAMA 274:1289, 1995). Because of the intimate involvement of lymphocytes and their products in the pathogenesis of MODS and their correlation with APBT mortality, we analyzed the relationship of lymphocyte recovery (day to recovery of 500 lymphocytes/cmm - ALC500) to OD during APBT. Thirty nine APBT patients were monitored daily for CNS dysfunction (drop of >4 points in the Folstein mini-mental status exam), pulmonary dysfunction (O2 saturation of <90% on 2 occasions >4 hours apart) and hepatic dysfunction (weight gain >5%, bilirubin >2 mg% and abdominal pain). The severity of illness score (SIS) was calculated by totaling the number of daily OD's (0-3) through the entire hospitalization. The ALC500 ranged from 9 to 75 days after beginning the preparative regimen (median 15 days). Fourteen patients experienced at least one OD during the transplant course. The first OD occurred before day 14 in all patients (range 1-13). In these 14 patients the SIS correlated with the ALC500 (R=0.665, p=0.01). Once OD had occurred, patients with more severe MODS had greater delays in lymphocyte recovery. When considering factors contributing to delayed immune reconstitution after APBT, severity of regimen-related MODS should be included. Conversely, the immune deficiencies associated with delayed immunologic reconstitution may be pathogenetically related to prior MODS.

29

OUTCOMES OF RELATED AND UNRELATED UMBILICAL CORD BLOOD (UCB) TRANSPLANT: A SINGLE INSTITUTION EXPERIENCE.

PR Haut, M Olszewski, M Kletzel. Children's Memorial Hospital, Chicago IL

Between January 1995 and February 1998 24 unrelated and 2 related UCB transplants were performed in patients (pts) with a median age of 6.8 years (range = 1.3-20.6). Diagnoses included hematologic malignancy (n=17), myelodysplastic syndrome (n=2), marrow failure (n=4), and immune deficiency (n=3). Median weight was 19.2 kg (range = 6.3-69.4). HLA disparity included: 0 antigens (n=2), 1 antigen (n=9), 2 antigens (n=11), 3 antigens (n=4, 2 related). Ablation for 21/26 pts was total body irradiation (TBI)(150 cGy BID x 4 days), cyclophosphamide (Cy)(60 mg/kg x 3 days) and Thiotepa (TT)(10 mg/kg) with etoposide (VP-16)(1000 mg/m2 continuous infusion x 2 days) added in patients with malignancy. Five patients received one of the following regimens: TBI/VP-16/TT (n=1), Busulfan/VP-16/TT (n=1), TT/Cy, TBI (150 cGy BID x 2 days)/Cy (n=1), and no ablation (n=1), GVHD prophylaxis consisted of continuous infusion cyclosporin, short course methotrexate, and anti-thymocyte globulin. Median total nuclear cell, mononuclear cell and $CD34^+$ cell dose infused was 8 x 10^8/kg, 0.4 x 10^8/kg, and 0.6 x 10^6/kg respectively. Median days to ANC > 500/(l was 26 (15-51) and platelets > 20,000/(l was 41 (16-100). Twenty-two pts had engraftment documented by VNTR, cytogenetics or HLA typing. Four pts died prior to documented engraftment but only one death occurred after the median time to engraftment for this group of pts. Fourteen pts had documented infections. Two pts had grade 4 mucositis requiring intubation. Eleven pts had grade 2 or less acute GVHD, 1 pt had grade 4 acute GVHD. Two patients developed chronic GVHD, one extensive. The one hundred-day mortality was 35%. Overall survival is 40% with a median follow-up of 766 days. Two of 10 patients with malignancies have relapsed. The described ablative regimen and GVHD prophylaxis for UCB transplantation resulted in acceptable rates of engraftment and GVHD given the degree of HLA disparity in this group of patients, however, the acute morbidity and mortality for the group was high. Modifications in the conditioning are being evaluated to reduce potential regimen-related toxicity.

30

COMPARISON OF CD4+ CTL EFFECTOR FUNCTIONS THAT MEDIATE SYNGENEIC GVL AGAINST DIFFERENT MYELOID LEUKEMIAS.

Michael Hsieh, Robert Townsend, and Robert Korngold

It is unclear whether and how GVL-mediating CD4+ T cells mediate direct antileukemic cytotoxicity *in vivo*. We sought to determine the role of perforin-, Fas ligand (FasL)-, and TNF-dependent mechanisms in the development of CD4+ T cell-mediated GVL activity against several myeloid leukemias.

These studies were performed using MMB1.10, MMB2.18, and MMB3.19; which are *myc*-transformed, mouse myeloid leukemias of C57B1/6 (B6) origin. Flow cytometric analysis revealed that all three tumors express high levels of cell surface Fas. RT-PCR detected mRNA for TNF receptor (TNF-R) type I and II in all three leukemias. Despite expression of TNF-R, none of the tumors were sensitive to TNF-mediated cytotoxicity as determined by MTT assays. For GVL mortality assays, B6 mice were lethally irradiated followed by challenge with tumor, and administration of either bone marrow alone or bone marrow in combination with tumor-presensitized CD4+ T cells. Use of wild type (w.t.), perforin-deficient (pfp^0), or FasL-deficient (*gld*) B6 donors revealed that CD4+ CTL rely equally on perforin and FasL in order to exert GVL activity against MMB1.10. Use of w.t., pfp^0, and *gld* donors in the MMB3.19 GVL model demonstrated that CD4+ CTL rely primarily on FasL and secondarily on perforin. An indirect role for NK cells in both GVL models was excluded through *in vivo* depletion of NK cells.

The differences in effector mechanisms utilized by CD4+ CTL against various leukemias does not appear due to intrinsic sensitivity of the tumors to perforin- versus FasL-mediated cytotoxicity; CD8+ T cells from pfp^0 donors are equally as poor at mediating GVL against MMB3.19 as CD4+ T cells from *gld* donors. We postulate that myeloid leukemias express tumor antigens that skew CD4+ CTL responses towards certain effector mechanisms over others.

31

CD34+ SELECTED BLOOD-DERIVED HLA-IDENTICAL ALLOGRAFTS FOR HEMATOPOIETIC MALIGNANCY IN OLDER PATIENTS: LOW RISK OF GVHD AND EARLY TRM.

TP Hughes, P Dyson, T Rawling, D Thorpe, C Rawling, N Horvath, G Dart, P Bardy, LB To. Division of Haematology, Hanson Centre for Cancer Research, IMVS, Adelaide, South Australia

Older patients receiving allografts have a high early transplant mortality (TRM) with GVHD being a major cause. A pilot study was undertaken in older patients (median age 51, range 41-60) with hematopoietic malignancies and HLA-identical donors using Filgrastim-mobilised blood cells which were CD34+ selected with the Isolex 300*i* device. Conditioning therapy was TBI 1200 cGy and cyclophosphamide 120 mg/kg. GVHD prophylaxis was cyclosporin only. Donors received Filgrastim 10 ug/kg daily (donors 1-9) or twice daily (donors 10-18) and were leukapheresed daily from day 4 until they achieved a target CD34+ cells dose of 5 million/kg recipient body weight after selection. Eighteen patients are enrolled - 4 CML, 2 ALL, 4 AML, 1 MDS, 3 Myeloma and 4 NHL (7 in early stage and 11 in advanced stage disease). Three of the first 9 donors and one of the second 9 donors mobilised less than 3 million CD34+cells/kg and unselected cells were also given; 2 of these patients died early of GVHD (1 case) or multiorgan failure (1 case). The remaining 14 patients received a median of 5.4 million CD34+ cells/kg (range 3.2-11.4) and 180,000 CD3+ cells/kg (3.5 log depletion). Neutrophils recovered to 500/ul by day 14 (range 10-22) and platelets reached 50,000/ul by day 15 (range 11-70). Acute GVHD was grade 0-1 in 11/13 patients and grade 2-3 in 2 cases. Four patients with leukemia who had only grade 0-1 GVHD were given 3 x 10^7 donor CD3+ cells/kg on day 60. One developed fatal grade 4 GVHD; 2 developed extensive chronic GVHD. TRM at 6 months was 20% in patients receiving only CD34+ selected cells and 33% for all patients. No patient has relapsed (median FU 11 months). We conclude that CD34+ selected blood allografts represent a promising approach in older patients, which might reduce the risk of GVHD and TRM.

32

G-CSF PRIMED BONE MARROW IMPROVES DELAYED ENGRAFTMENT PRODUCED BY MTX CONTAINING GVHD PROPHYLAXIS REGIMENS.

L. Isola, E. Scigliano, D. Skerrett and S. Frutchtman. BMT Service, Division of Hematology, Department of Medicine, Mount Sinai Medical Center, NY, NY, USA

Purpose of the Study: Administration of G-CSF leads to increased number of progenitors in harvested bone marrow. We previously demonstrated that G-CSF primed bone marrow (pBM) is a suitable source of stem cells for allogeneic transplantation[1]. The combination of methotrexate (MTX) and cyclosporin A (CSA) or tacrolimus (FK) is commonly used for GvHD prophylaxis after allogeneic transplantation. Prednisone and CSA or FK regimens results in more rapid hematopoietic reconstitution but steroids have undesirable side effects. We examined whether the use of pBM can offset the delayed engraftment seen with MTX containing GvHD prophylaxis. Methods: Seventeen patients received pBM from matched sibling donors who received G-CSF at 10 mcg/kg/day for 2-4 days prior to harvesting. Conditioning was TBI/CY, Bu/CY or TLI/CY/ATG. All grafts contained 3.5-4x10^8 MNC/kg. Ten out of 17 patients received MTX as part of their GvHD prophylaxis. IBMTR definitions of ANC>500 and plts>20,000 were used. Historical controls for engraftment were 112 consecutive pts. who received allogeneic BMT at our institution with unstimulated BM (BM). For length of stay, controls were the subset transplanted during 1996. Results: Neutrophil and platelet engraftment occurred more rapidly in both groups of patients receiving pBM as compared to controls and this shortened hospitalization (see table). Peritransplant mortality was 18% (3/17). Fourteen patients remain alive on day 57-1014 post-BMT. One patient in the pBM group who received CSA/PRD has extensive chronic GvHD. One who received MTX/CSA had an isolated CNS leukemic relapse and is now in CR.

Graft / MTX	ANC>500*	ANC>1,000*	Plt>20,000*	Hospital stay*
UBM / yes	24 (12	26 (13	26 (19	46 (15
PBM / yes	18 (3	21 (6	21 (5	34 (8
PBM / no	14 (4	17 (3	20 (5	36 (10

*Mean (SD

Conclusion: the use of primed bone marrow allografts resulted in more rapid engraftment and shorter hospitalization in this group of patients. Bone marrow priming can partially offset the delay in neutrophil and platelet recovery seen with the use of MTX containing GvHD prophylaxis. G-CSF pBM transplants didn't result in higher than expected GvHD, disease relapse and/or peritransplant mortality.

Isola, L.M., Scigliano, E., Skerrett, D., Shank, B., Ross, V, Najfeld, V., Fruchtman, S Bone Marrow Transplantation 20:1033-1037, 1997.

33

PHENOTYPE AND FUNCTION OF GRAFT-VS-TUMOR CONFERRING CELLS IN A MOUSE LYMPHOMA MODEL.

M Ito and JA Shizuru Stanford University Medical Center, Div. of Bone Marrow Transplantation, Stanford, CA

In prior studies we showed in a mouse B cell lymphoma model, BCL_1 that transplantation of highly purified MHC-mismatched hematopoietic stem cells (HSCs:Thy-1 ^{lo}Lin$^{-/lo}$Sca-1$^+$) results in 100% tumor relapse whereas transplantation of whole BM confers significant GVT. Here we used the BCL_1 model to study the phenotype and function of cells with GVT activity, and to study if MHC-matched BM can confer similar tumor protection. For the MHC-mismatched studies recipient mice were BALB/c (H-2^d, Thy 1.2) and donors were C57BL/Ka(BA:H-2^b, Thy1.1). Recipients were inoculated with 10^3 BLC_1 cells 1 wk prior to receiving lethal irradiation (XRT) and rescue with either whole BA BM, BA HSCs only, or BA HSCs plus candidate GVT conferring cells purified from BA BM (sorted CD3$^+$, CD4$^+$, CD8$^+$, Mac1$^+$, B220$^+$ or NK1.1$^+$ cells). No tumor protection was noted in mice transplanted with BA HSCs only (n=15); BA HSCs +Mac1$^+$ cells (n=5), +CD4$^+$ cells (n=8), +B220$^+$ cells (n=5), or +NK1.1$^+$ cells (n=10). In contrast, significant protection without GVHD was noted in mice transplanted with BA HSCs+CD3$^+$ BM cells (60%, n=5), +CD8$^+$BM cells (88%, n=8), or control BA BM (50%). CD3$^+$ and CD8$^+$BA spleen cells were also protective, however, CD3$^+$ spleen cells caused GVHD. To study the mechanism of the GVT effect, BALB/c received BCL_1 1 wk prior to receiving XRT and rescue with BM from immune defective knockout (K.O.) mice (perforin, IL-2, IFN-() or Fas-ligand defective mice. All donors were of the C57BL/6 background. All perforin K.O.(n=10) and Fas-ligand defective (n=10) BM transplanted mice died of tumor within 10 wks post-transplant. 100% of IFN-(K.O.(n=7) BM transplanted mice were tumor free but died within 10 wks post transplant, and IL-2 K.O. BM transplanted mice (n=15) died of a mixture of engraftment failure and tumor. To test if MHC-matched BM has measurable GVT activity, BCL_1 inoculated BALB/c (H-2^d, CD45.2) were transplanted with HSCs or BM from B10.HZ(H-2^d, CD45.1). 1 wk after inoculation the mice were irradiated and rescued with B10.HZ HSCs or BM. All HSC(n=10) and BM(n=10) transplanted mice died of tumor within 5-6 wks post-transplantation. Addition of splenic B10.HZ CD8$^+$cells resulted in significant GVT without GVHD, and B10.HZ CD3$^+$cells conferred GVT although at a reduced level compared with CD8$^+$cells. These studies suggest that in this model of lymphoma relapse CD8$^+$cells confer significant GVT without causing GVHD in both MHC-mismatched and MHC-matched strain combinations, and that tumor protection is dependent upon intact granule mediated cytolytic and Fas pathways. IL-2 appears to contribute weakly to tumor protection while IFN-('s role appears to be minimal.

34

RAFT VERSUS HOST DISEASE (GVHD) WITH GRAFT VERSUS MYELOMA EFFECT POST AUTOLOGOUS TRANSPLANT.

N. Janakiraman, C. Kasten-Sportès, S. Burt, I. Vial, J. Rogers, V. Ford-Cattaneo, V. Pindolia, Henry Ford Health System, Detroit, Michigan

The graft versus tumor effect of allogeneic bone marrow transplantation is being increasingly recognized, prompting attempts at reducing cytotoxic therapy and adding donor lymphocyte infusions (DLI). There are a few reports of benefits from DLI in patients with relapsed multiple myeloma showing graft versus myeloma effect. In a disease associated with high mortality from allogeneic transplantation, here is an opportunity to increase the therapeutic index. We report a patient with relapsed multiple myeloma who developed GVHD post autologous stem cell transplant and achieved a complete remission.

JM was a 61 year old male when he was diagnosed to have Stage IIA, IgA lambda, multiple myeloma. Refractory to VAD X 8 and Melphalan/prednisone X 8, in 5/93 he received Melphalan 140 mg/m^2 and fractionated TBI 960 cGy, followed by autologous Cytoxan-mobilized stem cells. He achieved a very good partial remission, with reduction in IgA levels from 4910 mg/dl to 600 mg/dl; however, there was a persistent monoclonal peak. Neupogen-mobilized stem cells were harvested during this remission and stored.

Patient relapsed in early 1998 and received Melphalan 200 mg/m^2 followed by 7.21 X 10^8 mononuclear cells/kg (CD34, 1.01 X 10^6/kg). About 4 weeks after infusion patient developed generalized erythematous rash consistent with GVHD of the skin. Skin biopsy at 3 months confirmed grade 3 GVHD. Interestingly, his monoclonal protein could not be detected at that time, even by immunoelectrophoresis. He was treated with steroids, with improvement of the skin rash; however, he had poor engraftment and died four months later of sepsis.

This case suggests the therapeutic benefit of GVHD in myeloma, which can be realized both in the autologous and allogeneic setting.

35

IPOTENTIAL FOR CLINICAL APPLICATION OF THE T-CELL BYPRODUCT FROM ELUTRIATION T-CELL DEPLETION AS PROPHYLACTIC DONOR LEUKOCYTE INFUSION.

W.E. Janssen, R.C. Smilee', M.J. Farmelo', K.K. Fields, S.C. Goldstein. Blood and Marrow Transplant Program, H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL.

T-cell depletion (TCD) from marrow allografts has been shown to reduce the incidence and severity of graft versus host disease (GvHD), albeit with increased risk for graft failure and/or relapse of disease. The risk of graft failure appears to diminish with minimal T-cell content in the graft and relapsed disease has been effectively treated in some cases with infusion of 10^6-10^8 donor T-cells/kg. The latter requires additional cell collection from the donor. For patients at high risk for severe acute GvHD, we have performed TCD using counterflow centrifugal elutriation (CCE) and CD34 positive selection. This approach has the unique advantage of retaining functional depleted T-cells for insuring minimum T-cell content in the graft and for subsequent donor leukocyte infusion (DLI) for relapse and/or prophylaxis. Following CCE, the small cell fractions are pooled and processed using the Ceprate™ CD34 cell selection system. The large cell fraction from CCE and the CD34$^+$ cell fraction from CD34sel are given to the patient. Additionally, sufficient cells from the T-cell rich CD34 negative cell fraction from CD34 selection are infused to achieve a final target CD3+ cell dose of 0.5x10^6/kg. The unused, T-cell rich, CD34neg fractions (T-cell byproducts) are cryopreserved for future use. Data from 12 patients undergoing TCD is summarized:

MEDIAN(RANGE)	MNC X10^6/KG	CD34+ X10^6/KG	CD3+ X10^6/KG
Start Marrow	**451 (289-964)**	**4.6 (0.9-10.4)**	**51 (17-65)**
Tot. Infused	**43 (6.0-118)**	**0.8 (0.3-3.1)**	**0.8 (0.4-5.1)**

The median days to achieve an ANC>500 for these patients was 13.5, exclusive of two graft failures. To determine the potential suitability of the frozen T-cell byproducts for DLI, they were analyzed, revealing:

MNC X10^6/KG	CD34+ X10^6/KG	CD3+ X10^6/KG	CD4/CD8 RATIO
34.4 (7.3-98.3)	**0.3 (0.06-1.4)**	**17.4 (2.9-27.4)**	**1.2 (0.3-3.8)**

We have also observed NK cells and both macrophage and dendritic lineage antigen presenting cells in the T-cell byproducts. These data compare with DLI products that may be obtained from donor leukopheresis. Our results suggest that the CCE + CD34 selection yields a functional graft and also a T-cell byproduct which may be used clinically for DLI. This may be particularly advantageous in unrelated marrow transplant, wherein access to donors subsequent to original harvesting and transplant is not assured.

36

GVHD INDUCED BY DOUBLE CYTOTOXICALLY DEFECTIVE DONOR T CELLS: OTHER EFFECTOR PATHWAYS CAN INDUCE GVHD WITH NOTABLY REDUCED EFFICIENCY.

Z. Jiang, N. Altman, M. Jones, E. Podack and R.B. Levy. Dept. of Microbiology and Immunology. Univ of Miami School of Medicine, Miami, FL 33101

To investigate effector mechanisms involved in the development and pathogenesis of graft-versus-host disease (GVHD), allogeneic bone marrow transplants (BMT) were performed with cytotoxically defective donor T cells. T cells from C57BL/6(H-2^b) Perforin/FasL double-deficient mice (B6-CDD) together with C57BL/6 Ly5^a congenic bone marrow were transplanted into MHC class I/II mismatched lethally irradiated BALB/C (H-2^b) recipients. Compared to the recipients of 0.5x10^6 wild type B6 T cells, BALB/C mice transplanted with 10X higher number (5x10^6) of B6-CDD T cells exhibited similar clinical signs of GVHD, including body weight loss, diarrhea, skin lesions and hair loss. Mice receiving 0.5x10^6 B6-CDD T cells exhibited a delayed and milder disease process. The number of spleen cells in long term surviving recipients of B6-CDD T cells, while initially higher vs spleen numbers B6 T cell transplanted mice, began to decrease within two weeks and diminished to the same number as in the recipients of syngeneic T cells. No evidence of lymphoproliferation or pancreatic and uterine infiltration by lymphocytes and monocytes/macrophage characteristic of CDD syndrome were identified in any BMT recipients. Mortality was observed in 60% and 40% of 5x10^6 and 0.5 × 10^6 B6-CDD (BALB/C recipients, respectively. Notably, 4 months post-BMT, analysis of recipient spleen and lymph node cells in B6-CDD T cell transplanted mice revealed that >95% of cells were K^b+Ly5^a+, indicating they were derived from the donor bone marrow Innoculum. PCR and phenotypic analysis demonstrated some CDD cells still persisted (~3%) in these spleens of the recipients. In total, the observations demonstrated: 1) Perforin/FasL double deficient T cells were capable of causing GVHD, however, comparable kinetics required 10X greater T cell numbers, and 2) allogeneic bone marrow was able to engraft when transplanted together with B6-CDD donor T cells. Notably, CDD T cells caused severe skin damage and hair loss, pathogenic processes not previously observed following BMT with FasL defective *gld* T cells. Further studies to understand these differences are being performed.

37

CHARACTERIZING THE EPITHELIAL INFILTRATION OF TCR V(FAMILIES IN A CD4+ T CELL MEDIATED MURINE MODEL OF ACUTE GRAFT-VERSUS-HOST DISEASE.

Stephen C. Jones, Thea M. Friedman, Debbie Statton, Mark Berger, George Murphy and Robert Korngold Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

C57BL/6By (B6) CD4+ T cells respond to minor histocompatability antigen (miHA) disparities in the BALB.B mouse. Aside from providing help for a lethal alloreactive CD8+ T cell response, they are alone capable of causing death in this murine model of graft-versus-host disease (GVHD). This research focuses on the latter of the two responses and is principally concerned with molecularly characterizing the responding B6 CD4+ T cell population and correlating this with expanding T cells identified directly at a GVHD target tissue site. CDR3 size spectratyping, a PCR based technique, was used to identify biases in the B6 CD4+ TCR V(repertoire once transplanted into lethally irradiated BALB.B recipients. Skewing of the CDR3 size distribution for a particular V(is suggestive of clonal or oligoclonal minor antigen driven expansion of that V(. Immunohistochemical analysis on epithelial tissue, a classical GVHD target organ, was performed with a panel of monoclonal antibodies specific for a number of V(families. We noted a larger infiltration in the BALB.B lingual epithelial tissue of V(+ cell populations which were skewed in the CDR3 size spectratype analysis compared to CD4+ T cells expressing a V(that did not show a biased CDR3 size representation. Furthermore, we directly observed a representative skewed V(expressing T cell population involved in a classical acute GVHD lymphocyte reaction pattern. These results demonstrate that the expansion of V(families as identified by spectratype analysis correlates with the pathogenesis of GVHD.

38

TREATMENT OF REFRACTORY ACUTE AND CHRONIC GRAFT VERSUS HOST DISEASE (GVHD) WITH ANTI TAC ANTIBODY (ZENAPAX®): EFFECTS ON ACTIVATED T CELLS AND IN VITRO IMMUNE FUNCTIONS.

N Kapoor, K Weinberg, M Smorswensky, G Crooks, D Kohn, R Parkman, Childrens Hospital Los Angeles, Los Angeles, CA

Nine patients between the ages of 3.5 and 17 years (8 recipients of unrelated bone marrow and 1 recipient of maternal bone marrow) developed graft versus host disease which progressed in spite of standard GVHD therapy. Four patients had refractory acute GVHD, and five others had refractory chronic GVHD. Three patients with acute GVHD had only skin involvement, and one had both skin and gut involvement. One patient with chronic GVHD had only liver involvement, and 4 patients had both skin and gut involvement. All the patients had previously received standard graft versus host disease prophylaxis and subsequently additional therapy with cyclosporine or FK 506, steroids, antithymocyte globulin, mycophenolate mofetil and/or PUVA. All the patients had failed to achieve clinical resolution or improvement of their GVHD.

All patients were treated with Zenapax while receiving their other current immunosuppressive therapy. All the patients received the drug at a dose of 1 mg/kg/dose given on Days 1 and 4 and then weekly doses for a total of five doses in six patients and six doses in three patients. All patients had immunological evaluations including peripheral blood immunophenotyping and T lymphocyte blastogenic responses to mitogens and antigens. Eight of nine patients had either complete or partial resolution of their GVHD processes. One patient died four weeks following the completion of treatment due to overwhelming pulmonary infection due to multiple bacterial, fungal and viral microorganisms.

Prior to the treatment with anti-Tac antibody, all the patients had a high the percentage of CD25+, CD26+ and DR+ T lymphocytes. Following treatment there was a marked decrease in CD25+ cells without significant alteration in percentage of CD26+ and DR+ cells. However there was a complete abrogation of T lymphocyte blastogenic responses to both mitogens and antigens. The exact time when these blastogenic responses will recover following the completion of anti-Tac antibody therapy and what will be the effect of the recovery of immunological function on the patients' clinical graft versus host disease status is under active study.

39

PHARMACOKINETICS OF ORAL AND I.V. MYCOPHENOLAT MOFETIL (MMF) IN PTS AT HIGH RISK FOR GVHD.

W Willenbacher[1], V Armstrong[2], AM Hartert[1], M Oellerich[2], M Shipkowa[2], N Basara[1], IW Blau[1], AA Fauser[1], MG Kiehl[1]. [1]Center for BMT & Hematology, Idar-Oberstein, [2]Dep. of Clinical Chemistry, Georg-August University, Göttingen; Germany

GvHD is a leading cause of morbidity and mortality post-BMT, especially in the setting of MUD and/or HLA-mismatched TX. One strategy to address this issue is the use of alternative immunosuppressive agents such as MMF. As shown previously[1] pre-dose blood levels of the main metabolite MPA are low in the early post-BMT phase if MMF is given p.o., (although a therapeutic range in the BMT setting has yet to be defined). A prospective study of the pharmacokinetics of a 2 x 1 g/d i.v. MMF schedule was instituted in pts. at high risk for GvHD (HLA mismatched VUD BMT) given from day + 10 onwards in addition to a standard immunosuppressive regime. Measurements of MPA were performed by EMIT and HPLC assays[2]. In contrast to the oral application of MMF higher blood levels of MPA were achieved with i.v. MMF; although the $AUC_{0\text{-}12h}$ was still well below values in organ TX (Media 20 mg x h/L vs. 40 mg x h/L). No second peak after 6h as in p.o. administration was observed. Clinical response was documented in several pts. switching from p.o. to i.v. MMF. Additional data on pharmacokinetics and clinical effectiveness of i.v. MMF will be presented.

References:
1 Hartert et al., BMT 1998 (submitted),
2 Schütz & Shipkowa, Trans Proc 30: 1185, 1998

40

DIFFERENTIAL INFECTION OF DIVIDING LYMPHOCYTES BY RETROVIRAL VECTORS ENCODING AN IMMUNOLOGICALLY SELECTABLE MARKER PERMITS PREFERENTIAL ENRICHMENT OF VIRUS-SPECIFIC T CELLS EARLY AFTER IN VITRO SENSITIZATION.

G. Koehne, H.F. Gallardo, M. Sadelain and R. J. O'Reilly. Bone Marrow Transplantation Service, Depts. of Pediatrics and Human Genetics. Memorial Sloan-Kettering Cancer Center, New York, NY

Retroviral vectors are known to selectively transduce proliferating cells. We wished to test whether this property of retroviruses could be exploited to select for EBV-virus reactive T cells early in the course of in vitro sensitization of PBMC. Replicate cultures of peripheral blood mononuclear cells were stimulated in vitro with mitogens or with 6000cGy irradiated autologous EBV transformed B cells. At varying times post stimulation, one of the replicates was infected with the retroviral vector. For this purpose, we used a MLV-based dicistronic vector, termed NIT, encoding a mutant NGFR and herpes simplex virus-thymidine kinase (hsv-tk). Test cultures were transduced during maximum initial proliferation (day 4 and day 7 for PHA and EBV transformed cells, respectively, as assessed by ^{3}H thymidine incorporation), at a MOI 1-2 for 24 hours on fibronectin fragments. Under these conditions, we regularly achieved transduction of 20-40% of the CD3+-lymphocytes. Sorting by two-color FACS resulted in collection of >98% purified NIT+ cells and a separate NIT- fraction. In ^{51}Cr release assays, NIT+ T cells sensitized to autologous BLCL showed 70% specific lysis of the autologous BLCL, as opposed to 20% by NIT- T cells and 30% by unselected, non-transduced control cells at a ratio of 20:1. Lysis of allogeneic BLCL and K562 targets was below 5% in the positive fraction. Limiting dilution analyses showed an autologous EBV-BLCL specific T cell frequency of 1/5,778 in the NIT+ cell population, a frequency of 1/202,003 in the NIT- fraction and a frequency of 1/230,345 in the unmodified fraction, when the autologous BLCL were used for stimulation. The frequency against an allogeneic BLCL was <1/500,000 in the three cell fractions of this donor. Identically prepared T cells from another donor showed, in LDA, a frequency of 1/10,265 against autologous BLCL and 1/14,371 against the allogeneic BLCL in the unmodified T cells. In contrast, the NIT+ cells showed a frequency of 1/5,582 against autologous and 1/104,604 against the allogeneic BLCL. Transfer of a vector encoding an immunologically selectable marker into T lymphocytes early after sensitization permits multifold enrichment of EBV-specific T cells, while depleting of allo-reactive T lymphocytes at the same time. Thus, our genetic approach permits rapid selection and propagation of virus-specific T cells for adoptive therapy of EBV-associated disease.

41

B CELL POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER GENETICS: RECURRENT ABNORMALITIES DEFINED BY MOLECULAR CYTOGENETICS OF DNA IN T CELL DEPLETED BMT.

L.J. Krueger[1], M. Ladanyi[2], D.A.Filippa[2], R.J.O'Reilly[3] and S.P. Dunn[1] St. Christopher's Hospital for Children[1], Philadelphia, PA and Memorial Sloan-Kettering Cancer Center[2,3], New York, NY

B cell posttransplant lymphoproliferative disorders (BPLDs) are complications in bone marrow transplantation. They range from benign hyperplasia to frank lymphoma. Cytogenetic analysis of BPLDs is limited by the quality/quantity of the material, viability, mitotic activity, purity, heterogeneity between specimens, sensitivity and other related technical issues. While genetic analysis remains the keystone for the subclassification of the BPLD, limited progress in defining the genetic pathways has occurred. Comparative genomic hybridization (CGH), a global process defining quantitative genetic changes, was applied to a series of BPLD in BMT (n=16 pts). CGH identifies candidate areas of the genome facilitating the isolation of specific candidate genes.

16 BMT pts diagnosed with BPLD had an average age of 32 yrs (range 10 to 53 yrs) and a mean time to development of BPLD of 4 months (range 3-8 months). The BPLD biopsies were obtained from various tissues mostly showing diffuse large cell lymphoma. 93% of the specimens evaluated were positive for EBV; almost all showing EBV clonality. CMYC rearrangement was absent by Southern blotting (0/12). In a subset (n=9), CGH defined 22 subchromosomal or chromosomal gains (approximately 3 gains per patient) with 2 losses of the X chromosome. Five regions were recurrent including: 1p, 16p, 17, 19q and 21. The gain of chromosomal subregion 1p34.2p36 in these BPLD pts. has also been described as a marker for endemic, but not sporadic Burkitt's lymphoma (another EBV-associated lymphoma). In the CGH cases, the IGH rearrangement analysis confirmed the clonal nature of the biopsy and the donor origin was confirmed by donor-host polymorphism analysis. CGH provides a global technique for defining genetic lesions in the initiation and progression of BPLDs. CGH analyses in BPLDs may show genetic overlaps, improve discrimination between gene paths found in EBV-associated lymphomas, define new genetic lesions and refine the molecular analysis of lymphomagenesis in BPLDs.

42

PROPHYLAXIS OF GRAFT-VERSUS-HOST DISEASE (GVHD) WITH CYCLOSPORINE-PREDNISONE (CSP-PRED) IS ASSOCIATED WITH AN INCREASED RISK OF CHRONIC GVHD (cGVHD).

K.R.Shaji Kumar, M. Litzow, M. Chen, D. Gastineau, M. Gertz, D. Inwards, M. Lacy, A. Tefferi, Mayo Clinic, Rochester, MN*

We retrospectively reviewed the charts of 271 consecutive allogeneic related blood and marrow transplants (BMT) performed at our institution between 1982 and 1997 to determine the effect of two different GVHD prophylactic regimens, cyclosporine-short course methotrexate (CSP-MTX) and cyclosporine-prednisone (CSP-PRED), on the incidence of cGVHD. CSP-MTX or CSP-PRED were used in 198 (73%) patients (pts) whereas other regimens were used in the remaining 73 pts. Patients surviving <60 days post-BMT (n=30) were censored from the analysis for incidence of cGVHD leaving 120 pts (71%) in the CSP-MTX group and 48 (29%) in the CSP-PRED group. CSP-PRED was used in pts transplanted more recently because of concerns of an increased risk of veno-occlusive disease of the liver in pts receiving CSP-MTX. Marrow was the source of stem cells in virtually all pts. The two groups did not differ significantly with respect to age, sex, HLA match (5/6 vs. 6/6), stem cell source, diagnosis, or conditioning regimen. Chronic GVHD occurred more commonly in the CSP-PRED group (75%) than in the CSP-MTX group (43%) (p=0.0002). Among the 88 pts (52%) who developed cGVHD, 56(64%) had extensive cGVHD and 32(36%) had limited cGVHD. The incidence of extensive cGVHD was higher in the CSP-PRED group (52%) compared with the CSP-MTX group (26%) (p=0.001). Clinical manifestations of cGVHD were similar in both groups. The higher incidence of extensive cGVHD in the CSP-PRED group contributed to increased late mortality in this group, but the overall survival (n=198) did not differ significantly between the CSP-MTX (n=145) and the CSP-PRED (n=53) group. We conclude that the choice of prophylactic regimen for acute GVHD can impact the incidence of cGVHD and needs to be considered when comparing the incidence of cGVHD in studies analyzing transplants from different stem cell sources.

43

SINGLE LARGE VOLUME LEUKAPHERESES (LVL) AFTER 6 G/M^2 OF CYCLOPHOSPHAMIDE (CFF) ALLOW THE COLLECTION OF LARGE HAEMATOPOIETIC GRAFTS FOR AUTOLOGOUS TRANSPLANTATION IN MYELOMA.

F Leal-da-Costa, JL Passos-Coelho, A. Machado, N Miranda, MRG Silva, P. Lúcio, A. Parreira. Hematology, Portuguese Institute of Oncology, Lisbon, Portugal

Large PBPC collections potentially ensure faster haematopoietic recoveries and reduced complications and may allow safe ex vivo manipulations and/or double transplants. Single LVL are cheaper and more convenient for patients (pts) and for the transplant team allowing for resources optimization. Since 1995 we have been collecting PBPC with LVL after CFF (6g/m^2) in multiple myeloma pts candidates for autologous haematopoietic transplantation. Twenty one pts, median age 48(33-67) y.o., received CFF on day 1 and filgrastim 10(7.8-12) µg/kg/day starting on day 5. When mobilized 15 pts had less than one year after diagnosis and 9 had only one line of previous anti-myeloma treatment. One patient with pneumothorax, hemorrhagic cystitis and neutropenic fever was not discharged after high-dose CFF and 10/20 pts were readmitted because of neutropenic fever. In only 1 pt, with Rhodotorula rubra sepsis, was the harvest cancelled. There was no treatment related mortality. LVL were performed, as previously described(1), on day 14(13-18), when peripheral blood leucocyte count was 32(1.48-72.9) X 10^9 /L, platelet count was 81(39-150) X 10^9 /L and CD34+ count was 94(4-721) X 10^6 /L. With an average of 9.7(2.3 sd) blood volumes collected in 304 (59 sd) min, at a flow rate of 142(15 sd) ml/min, 8.6(6.41-13.76) X 10^8 nucleated cells /Kg, and 10(0.06-55.5) X 10^6 CD34+ cells/kg were collected. Fourteen pts had > 5 X 10^6 / CD34+ cells /Kg collected in a first single procedure. Three pts had a second mobilization with G-CSF and 1 pt had bone marrow harvested. So far, 27 transplantations were performed in 19 pts. In conclusion, PBPC collection with single LVL after mobilization with high dose CFF and G-CSF is an effective way to collect large PBPC grafts.

(1) J.L. Passos-Coelho et al, Journal of Hematotherapy 1997;6:465-474.

44

DOUBLE CYTOTOXIC DEFICIENT MICE PRIMED TO DONOR ANTIGENS CAN REJECT BONE MARROW ALLOGRAFTS.

R.B. Levy, M. Mammolenti, E. Podack and M. Jones. Dept. of Microbiology and Immunology. University of Miami School of Medicine. Miami, FL 33101

The rejection of marrow allografts can frequently occur in recipients of T cell depleted inoculum or in individuals who have been previously exposed to donor antigens. To examine the involvement of the two major cytotoxic pathways i.e., perforin dependent and FasL- dependent killing utilized by lymphoid cells in a marrow rejection model, we examined allografts transplanted into cytotoxic double deficient (CDD) recipients. Five week old B6-CDD and cytotoxic normal B6 (H-2^b) mice were primed against non-MHC allogeneic antigens by inoculation of C3H.SW (H-2^b) spleen, thymocytes and lymph node cells. Two weeks later, primed and unprimed (control) recipients were irradiated (9.0Gy TBI) and transplanted with T cell depleted marrow from C3H.SW or B6 (syngeneic) donors. Resistance was assessed by assaying spleen cells five days post-BMT for CFU-GM-CSF and CFU-IL-3 activity. Unprimed B6-CDD and unprimed normal B6 recipients contained high numbers of CFU's following transplant with 2x10^6 syngeneic B6 marrow. Unprimed B6-CDD recipients transplanted with 2x10^6 allogeneic C3H.SW marrow contained >150 and 120 CFU/culture GM-CSF and IL-3 respectively. B6-CDD primed and B6 primed mice also demonstrated CFU activity after BMT with syngeneic B6 marrow. However, primed B6-CDD recipients contained marginally detectable (<15 and <12) CFU-GM-CSF or CFU-IL-3 respectively after transplant with 2x10^6 C3H.SW TCD marrow. Transplant with 4x10^6 C3H.SW TCD marrow also failed to result in significant CFU activity (<10 CFU/culture) indicating strong resistance in the B6-CDD recipients. These results were indistinguishable from those which have been obtained after identical BMT into primed B6 recipients. The present results indicate that recipients simultaneously unable to mediate perforin and FasL dependent cytotoxicity can effectively generate resistance against progenitor cell engraftment. We conclude that a non-perforin and non-FasL dependent effector mechanism(s) can effect antigen specific marrow resistance in this model. Experiments are underway to examine for involvement of other death receptor/ligands and cytokines in this model system.

45

IN VITRO GENERATION OF CMV AND EBV SPECIFIC CYTOTOXIC T-LYMPHOCYTE CULTURES.

Q. Sun, R. Burton, L.J. Dai, K. Pollok and K. Lucas; Bone Marrow Transplantation Program, University of Alabama at Birmingham

Epstein Barr virus (EBV) and cytomegalovirus (CMV) infections are a serious cause of morbidity in patients receiving T cell depleted stem cell transplants. Since cytotoxic T-lymphocytes (CTL) play a critical role in controlling viral infections, the infusion of *in vitro* expanded, virus-specific CTL has been actively explored in therapeutic and prophylactic regimens for EBV and CMV infections. We attempted to generate EBV and CMV specific CTL cultures simultaneously using EBV-immortalized B lymphoblastoid cell lines (BLCL) as stimulators. BLCL and skin fibroblasts (SF) were transduced with a recombinant retrovirus that encoded the immunodominant CMV protein pp65 and a neomycin selectable marker. BLCL transduced with pp65 (pp65-BLCL) and skin fibroblasts (pp65-SF) stained positive for pp65 using immunoperoxidase staining and were positive by Western blot analysis. The pp65-expressing cells were co-cultivated with autologous peripheral blood mononuclear cells, and were tested for specific cytotoxicity against CMV and EBV in chromium release assays. The pp65-BLCL primed CTL cultures displayed specific cytotoxicity against pp65-expressing SF, pp65-BLCL, and CMV infected SF. When non-transduced BLCL and BLCL transduced with the vector alone were assayed, there was no difference in the amount of cytotoxicity. These findings suggest that the cytotoxicity of the pp65-BLCL primed CTL cultures was specific for CMV pp65 as well as for EBV BLCL, and that the selection marker bacterial neomycin phosphotransferase was not immunogenic. This CMV specific cytotoxicity appeared to be against pp65 only and was HLA Class I-restricted, since 1) similar cytotoxicities were observed against SF transduced with the pp65-encoding retrovirus or infected with a pp65-encoding vaccinia virus, but not the control targets, and 2) antibodies against HLA Class I but not HLA Class II inhibited more than 50% of the pp65 targeted cytotoxicity. Thus, our preliminary data suggested that this strategy might be used to generate bi-specific CTL cultures *in vitro*.

46

INDUCTION OF TRANSPLANTATION TOLERANCE ACROSS MAJOR AND MINOR HISTOCOMPATIBILITY BARRIERS USING NON-MYELOABLATIVE CONDITIONING WITH FLUDARABINE, LOW DOSE IRRADIATION, AND CYCLOPHOSPHAMIDE

Leo Luznik, Laura W. Engstrom and Ephraim J. FuchsJohns Hopkins Oncology Center, Baltimore, MD

Administration of cyclophosphamide (Cy) 2-3 days after cell transplantation has been shown by multiple investigators to induce tolerance and low-level (3-4%) chimerism across minor but not major histocompatibility barriers. In this model, (50 million donor cells must be administered before Cy for tolerance to be induced. The goal of the current experiments was to lower the cell dose threshold for the induction of tolerance and to facilitate the induction of tolerance across major histocompatibility complex (MHC) barriers. Treatment of animals with 200 cGy total body irradiation (TBI) prior to transplantation lowered the tolerogenic dose to 10 million cells and increased donor cell chimerism to 20% following the administration of Cy. However, this same regimen failed to induce tolerance in animals with major MHC antigen differences. Because of the apparent rejection of MHC-incompatible cells, we have added fludarabine to pretransplant irradiation to increase the degree of recipient immunosupression. DBA/2 mice treated with Fludarabine 60 mg/M^2 on d.-3 and -2, 200 cGy TBI on d.O and Cy 200 mg/kg IP on d.3 (Flu-XRT-Cy) accept minor + MHC antigen-incompatible B6-Ly5.2 marrow grafts given on day 0. Chimerism at 4 and 8 weeks was minimal but detectable with 10 million donor marrow cells, whereas 20 million donor marrow cells induced approximately 65% donor T cell chimerism within the spleen and thymus at 8 weeks after transplant. Addition of pre-transplant Cy to this regimen (to generate "Flu-Cy-XRT-Cy") further augmented chimerism within the spleen and thymus to >95%. Donor chimerism was detected in granulocytes and B cells as well. These data suggest that tolerance and significant chimerism with MHC and minor antigen-incompatible cells can be readily achieved by preparing recipients with a combination of fludarabine, cyclophosphamide and low dose irradiation. This regimen may be easily adapted for non-myeloablative transplantation using HLA-haploidentical donor-recipient pairs.

47

ANALYSIS OF GVHD FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION IN FASL DEFECTIVE AND NORMAL RECIPIENTS.

M. Mammolenti, M. Jones and R.B.Levy. Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101

We are interested in understanding the involvement of cytotoxicity following allogeneic bone marrow transplantation (BMT). To investigate if the expression of functional FasL in recipients can effect the outcome post transplant, MHC matched allogeneic BMT was performed using FasL defective (B6.*gld)* or FasL normal (B6) recipients. Varying numbers of unprimed C3H.SW T cells together with normal B6 TCD marrow was injected into 9.0Gy recipients. Mice were monitored for weight loss, survival and clinical signs of GVHD. Modest clinical changes were identified in both groups. Transient weight loss also occurred in both groups. In one experiment, B6.*gld* but not B6 recipients died after transplant with high numbers (4 x10^7) of C3H.SW T cells. Subsequently, T cells from C3H.SW (H-2^b) mice primed to B6 (H-2^b) antigens were transplanted. Again, the kinetics and amount of weight loss in the two groups were indistinguishable. No significant differences at 2 and 4 weeks post-BMT were apparent in the total numbers of cells in the thymus and spleen in B6.*gld* and B6 recipients. Phenotypic analysis demonstrated both groups of recipients had few DP thymocytes and an inverted splenic CD4/CD8 ratio as well as few B220+ cells in this tissue. Interestingly, skin involvement appeared to occur more rapidly in the FasL-defective B6.*gld* recipients. Following BMT with 1 x10^7 T cells, skin lesions appeared within 3-4 weeks in B6.*gld* but not normal B6 (>40 days) recipients. BMT using higher numbers of T cells again resulted in earlier and more severe skin changes in B6.*gld* recipients. Histological analysis of skin and other FasL expressing tissues is being examined. If host FasL expression post-BMT is important, B6.*gld* recipients might exhibit more rapid lympho-hematopoietic GVHD or target tissue destruction (e.g. skin, liver, GI) and/or perhaps additional (ex. testes, eye) tissue involvement. The present data suggest that other regulatory molecules or FasL expression by donor populations may be sufficient to regulate donor lymphoid cell expansion post-BMT. However, FasL expression in skin may be capable of inhibiting pathogenesis in this tissue.

48

EX VIVO EXPANDED BONE MARROW PRODUCT IN COMBINATION WITH LOW DOSE OF AUTOLOGOUS PBSC ENHANCES CLINICAL OUTCOME.

R.K. Mandalam, J. Douville, [1]P.J. Stiff, [2]A.J. Pecora, R.D. Armstrong, and A.K. Smith Aastrom Biosciences, Inc., [1]Loyola University Medical Center, [2]Northern New Jersey Medical Center

Infusion of ex vivo expanded autologous bone marrow (BM) cells as the sole source of rescue in myeloablative STAMP V chemotherapy treated breast cancer patients results in short- and long-term hematopoietic and immune reconstitution (Stiff et al., Blood 90:395a, 1997). BM cells obtained from a small volume aspirate ((40 mL) expanded using cytokine (PIXY321, Epo, Flt3-L) supplemented medium and continuous perfusion results in a) formation of a stromal layer providing a hematopoiesis supporting microenvironment b) expansion of stem and progenitor cells, and c) passive purging of tumor cells. An automated clinical scale system called the AastromReplicell™ Cell Production System has been developed to implement the biology of the ex vivo expansion process under cGMP conditions. More recently, ex vivo expanded BM cells generated (using conditions described above) from a cryopreserved/thawed small volume whole BM sample ((1.5 x10^9 viable cells) were evaluated clinically in combination with a suboptimal PBSC CD34+ dose. In a pilot study, breast cancer patients (Stage II-IV) received 10 (g/kg/day of G-CSF for 5 days with a small volume of BM collected for expansion on day 3. The patients then went on to apheresis for PBSC collection with subsequent administration of the STAMP V regimen. Eight patients receiving the 0.5 x10^6 PBSC CD34+ cells/kg + AastromReplicell System expanded BM cells reached ANC (500 and platelets (20,000 on median days of 10.5 and 13 respectively. Seven patients receiving (0.5 to 2.0 x 10^6 PBSC CD34+ cells/kg + AastromReplicell System expanded BM cells reached ANC (500 and platelets (20,000 on median days of 10 and 11 respectively. The period to reach these clinical recovery thresholds are significantly superior, particularly for platelet recovery, than those reported for a similar dose of CD34+ PBSC alone. Additionally, patients undergoing transplant with the combination of PBSC and Aastrom Replicell expanded BM cells showed a median of 2 febrile days compared to the typical 4-6 febrile days seen with PBSC transplants at these clinical sites. Use of the AastromReplicell System in combination with suboptimal PBSC CD34+ doses has the potential to yield optimal recoveries for patients mobilizing poorly while also minimizing the risk of reinfusion of contaminating tumor cells by both passive purging achieved during the BM expansion process and reduction in the apheresis product as only low dose of CD34+/kg is required.

49

INFILTRATING T-CELLS DURING LIVER GVHD SHOW A RESTRICTED T-CELL REPERTOIRE.

D. Margolis, T. Janczak, L. McOlash, and J. Gorski. Medical College of Wisconsin, Milwaukee, WI

Data from animal models has shown that hepatic graft versus host disease may be mediated by donor T cells interacting with liver adhesion molecules and/or other minor histocompatibility antigens. We hypothesized that T-cell infiltrates within a liver biopsy during clinical GVHD would show a restricted T-cell response because the T-cells would be responding to a limited number of antigens. The peripheral T-cell repertoire and the liver infiltrating T-cell repertoire was studied in a patient who initially developed skin graft versus host disease with subsequent development of liver GVHD after a matched sibling BMT for AML. Spectratype analysis of the peripheral blood at the time of liver GVHD revealed that the patient had reconstituted a complex peripheral T-cell repertoire as evidenced by CDR3 length heterogeneity presence in most of the T-cell families. The repertoire complexity was skewed to one CDR3 length predominating in VB 5.3, 4, 7, 8, 15, and 6.1. Spectratype analysis on the liver biopsy sample revealed a limited infiltrate with an oligoclonal expansion in VB's 2, 4, 7, 8. The T-cell infiltrate was evaluated in more detail by sequencing the relevant expansions noted by spectratype and then developing probes to the predominant CDR3 sequences found (clonotype). The clonotype probes were hybridized to the peripheral blood and liver samples from the patient, a T-cell line developed using T-cells in the peripheral blood at the time of the initial skin GVHD, the donors' blood and marrow, and controls. The results are: (A) The oligoclonal expansion in the blood in families VB15 and VB5.3 were derived from the donor, are present in a T-cell line derived from the patient at the time of the initial skin GVHD, but are not found in the liver; (B) The oligoclonal expansion found in the liver in VB 7 and VB 4 were derived from the donor, are *not* present in a T-cell line derived from the patient at the time of the initial skin GVDH and are present in the liver and the peripheral blood. These results show that the T-cell infiltrate during liver GVHD is mediated by a limited number of T-cells. These cells are different than T-cells expanded in the peripheral blood during an acute skin GVHD reaction. This data is evidence supporting the hypothesis that liver GVHD is a response to tissue specific minor histocompatibility antigens. Further work must be done to define these antigens.

50

SYNGENEIC LYMPHOCYTE INFUSION (SLI) FOLLOWING ALLOGENEIC BMT RESULTS IN A DELAY OF CLINICAL GVHD AND PROLONGED RECIPIENT SURVIVAL: A MODEL FOR GVHD RESCUE.

L. Marks and R.B. Levy. Dept. of Microbiology and Immunology.University of Miami School of Medicine, Miami, FL 33101

Allogeneic bone marrow transplant (BMT) is the treatment of choice for a wide variety of hematopoietic disorders, including malignancies. Graft-versus-host disease (GVHD) is currently the second most significant cause of morbidity and mortality following an allogeneic BMT. Experimental, as well as clinical allogeneic BMTs involving donor-recipient MHC Class I/II disparities result in acute GVHD. To determine if it is possible to rescue recipients from GVHD, we examined the efficacy of a syngeneic lymphocyte infusion (SLI) of BALB/C cells post-BMT in the B6 ($H\text{-}2^b$) (BALB/C ($H\text{-}2^d$) BMT model. BALB/C mice were irradiated with 8.25 Gy, and the following day B6 bone marrow and 0.5 x 10^6 T lymphocytes were innoculated by i.v. injection. The following groups of BALB/c cells (spleen and lymph node cells that were either naive, or primed *in vivo* against B6) were infused at day 13 post-BMT: A-no cells (vehicle only); B-100 x10^6 primed cells, C-50 x10^6 primed cells; D-100 x10^6 naive cells; E-30 x10^6 primed cells that had been T-cell depleted (TCD), and F-50 x10^6 primed cells that had been T-cell enriched (TCE).

Survival at day 50 post-BMT was as follows: A-0%, B-100%, C-0%, D-50%, E-0%, and F-25%. Mean survival time was >63 days in Gr.B, compared to 40 days in Gr.A. Notably, the average weight of Gr.B mice stabilized beginning at day 11 post-SLI, whereas Gr.A mice experienced perpetual weight loss. Additionally, Gr.B mice showed a prolonged delay before the onset of severe symptoms characteristic of GVHD (fur ruffling, haunched posture, diarrhea) with an average time of onset of 56 days compared to the Gr.A average time of onset of 36 days. The results have demonstrated that SLI can induce an early increase in survival, and delay the onset of the clinical GVHD process. We plan to pursue these results by examining the mechanism of rescue and the role of timing of the SLI in this and other BMT model systems.

51

DONOR-DERIVED HEMATOPOIESIS IN LONG-TERM MARROW RECIPIENTS.

Mathioudakis,G., Storb, R., McSweeney, PA, Gass, MJ, Lansdorp, PM, Brummendorf, TH, Flowers, MED and Nash, RA. Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA and Terry Fox Labs, Vancouver, BC.

Here we characterize donor-derived hematopoiesis in long-term (> 20 yrs) allogeneic marrow recipients. Clinical status of hematopoiesis was determined from complete peripheral blood counts (CBC) and by chimeric analysis using Y-chromosome fluoresence in-situ hybridization (FISH) or microsatellite markers. Clonal status was examined by X-linked clonal analysis using probes for the PGK and M27(genes and telomere length by terminal restriction fragment (TRF) and by Flow-FISH analysis. At the time of study, CBC were normal in all patients except one, who was pancytopenic with high mean corpuscular volume (MCV), absolute neutrophil count (ANC) of 400, platelet count of 40,000 and hematocrit of 34%. All but one patient, who had mixed chimerism, were full chimeras (>96% donor), including the patient with pancytopenia. We found that the recipients had shorter telomeres ((TRF) compared to their donors, including the patient with pancytopenia, who in fact had the highest degree of shortening ((TRF=1.7 kb). All patients with female donors had polyclonal hematopoiesis as determined by X-linked analysis. The first patient identified with pancytopenia is the third longest survivor of an allogeneic transplant at FHCRC, 27 yrs after transplant for aplastic anemia (AA). He very recently received purified CD34+ cells from his initial marrow donor. Very recently an additional patient with late pancytopenia has been identified. He was transplanted more than 20 years ago for AA and whereas his CBC was normal until recently, he developed pancytopenia with a high MCV and normal B12 and folate levels. Our findings indicate that the majority of long-term marrow recipients have normal counts, polyclonal hematopoiesis and accelerated telomere shortening. There are, however, at least 2 long-term recipients with late donor-derived marrow failure and marked telomere shortening, raising the issue of late marrow dysfunction and potential correlation with telomeric loss. We aim to expand our studies to include more patients and by estimating their stem cell pool reserve by marrow cultures.

52

PROSPECTIVE NEUROCOGNITIVE ASSESSMENT FOLLOWING BONE MARROW TRANSPLANTATION: PART I. CHILDREN.

McCabe, MA, Shimoda, KC, Schuman, WB, Dinndorf, PA, Children's National Medical Center and George Washington University School of Medicine, Washington D.C.

Bone marrow transplantation (BMT) includes preparative regimens (i.e., total body irradiation, high dose chemotherapy) which may be toxic to the developing nervous system. Prospective cognitive and achievement testing has been analyzed for 25 survivors ((3 years of age) at 18-months, 19 survivors at 3-years, and 10 survivors at 5-years post-BMT in relation to their baseline functioning. Results suggest that BMT regimens in childhood may indeed be associated with decline in cognitive and achievement scores for some children. Non-parametric analyses suggest that risk factors identified in the literature on neurocognitive outcome with childhood malignancies (i.e., diagnosis of ALL, previous craniospinal radiation) also play a role in neurocognitive outcome following BMT.

53

PLATELET TRANSFUSIONS: UTILIZATION AND ASSOCIATED COSTS IN A TERTIARY CARE HOSPITAL.

K.R. Meehan[1], C.O. Matias[1], S. Rathore[2], S.G. Sandler[1,3], J. Kallich[4], J. LaBrecque[4], M.H. Erder[4], K.A. Schulman[2]. [1]Bone Marrow Transplant Program, [2]Division of Clinical Economic Research Unit, [3]Blood Bank, Georgetown University Medical Center, Washington, DC 20007, [4]Amgen, Inc., Thousand Oaks, CA

We performed a prospective study to evaluate the utilization of platelet transfusions and compare resource use and costs among all admitting services and diagnoses in a tertiary care hospital for a 6-month period. Inpatients receiving platelet transfusions between July and December 1996 were followed prospectively. Clinical and financial data were collected, categorized and crosschecked according to patient admitting service and principal diagnoses using ICD-9 codes. During the study period, 1,957 platelet transfusions were administered to 247 inpatients. Seventy-five percent of all platelet transfusions were single donor (apheresis) units. Of 1,468 single donor platelet units and 489 random donor platelet units transfused, 86% were administered to bone marrow transplant (BMT) patients and patients with hematological malignancies/ diseases. These two admitting services accounted for 61.5% of all hospital inpatient costs during this time period. Of 247 inpatients who received platelet transfusions, 51 became refractory to platelet transfusions (20.6%); BMT patients (n = 29) and Leukemia/Lymphoma patients (n = 14) comprised the majority of these patients. Platelet-refractory patients received 57.4% and 69.2% of all random and single donor platelet transfusions. On average, the hospital stay was longer (33.3 days versus 14.5 days) and inpatient resource use and hospital costs were more than three times higher for patients refractory to platelet transfusions. Platelet-refractory patients use significantly more single donor platelets and incur more hospital costs than nonrefractory patients. Strategies that would decrease the use and costs of platelet transfusions include lowering the threshold to 10,000(L for the administration of prophylactic platelets and the increased use of random donor platelet transfusions respectively.

54

IMPACT OF TRANSPLANTATION ON THE DELIVERY OF LOCAL RADIATION IN HIGH RISK BREAST CANCER.

HCF Moore, R Mick, C Sickles, P Mangan, L J Solin, E Glatstein, G Buzby, KR Fox, SM Luger, DL Porter, LM Schuchter, E Lob, S Schuster, L Silberstein, EA Stadtmauer U of PA Cancer Center; Philadelphia, PA

High-dose chemotherapy with stem cell transplant is used increasingly in the treatment of breast cancer. Because even high-risk patients may be cured with surgery, conventional adjuvant chemotherapy and local radiation, it is important to assess the interaction of transplant with the delivery of standard aspects of treatment. Between June 1992 and December 1997, we treated 103 women with high dose Cytoxan and Thiotepa and stem cell support for stage II or IIIa breast cancer involving (4 axillary lymph nodes following surgery and conventional-dose adjuvant chemotherapy. Disease-free and overall survival rates at the median follow-up time of 18 months were 77% (+/-4%) and 83% (+/-4%) respectively. Twenty patients (19.4%) had undergone local radiation therapy prior to transplant. Of the remaining 83 patients, 6 did not receive radiation therapy because of thrombocytopenia (3 patients), transplant toxicity (2 patients) or disease progression (1 patient). Median time from definitive surgery to completion of radiation therapy was 34.6 weeks (range 10.9 to 50.1 wks.) for patients irradiated prior to transplant and 43.4 weeks (range 26.1 to 76.1 wks.) for patients irradiated after transplant. The mean radiation dose delivered was 5328 cGy. Sites of first relapse were known for 26 of 27 patients who progressed; of these, 5 had locoregional recurrence alone as their first site of failure. For patients irradiated prior to transplant, 3 of 7 (43%; 95% CI 6% to 80%) first recurrences were local, while 2 of 19 (10.5%; 95% CI 0% to 24.5%) first recurrences were local alone in patients for whom radiation was delayed or omitted. While tumor characteristics were similar in the two groups, no increase in the relative rate of local recurrence was observed in patients who did not receive early radiation. No difference in disease-free survival was seen between the two groups of patients. Deferring radiation therapy until after transplant does not appear to adversely impact patterns of recurrence or disease free-survival.

55

CHARACTERIZATION AND OUTCOME OF "HARD TO MOBILIZE" LYMPHOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION.

M. Sugrue, K. Williams, S. Peracha, S. Khan, J.R. Wingard, J.S. Moreb. University of Florida, Gainesville, FL.

A "hard to mobilize" patient was defined as one in whom ($1x10^6$ CD34+ cells/kg cannot be obtained after two consecutive large volume apheresis. In the present study, data from all lymphoma patients, Hodgkin's and non-Hodgkin's, enrolled consecutively for treatment between June 1996 and June 1998 were analyzed. A total of 44 patients were studied and 21 (48%) met the definition of "hard to mobilize" (Group I). Main differences between the "good mobilizers" (Group II) and Group I were in the median number of prior treatments (2 vs 3, respectively) and the frequency of radiotherapy use (32 vs 47%, respectively). Of Group I, 7/21 (33%) patients were unable to achieve a minimal dose of ($1x10^6$ CD34+ cells/kg even after a second cycle of apheresis and/or bone marrow (BM) harvest (n=5). Overall, 11/21 (52%) required an additional mobilization and/or BM harvest. Only 3/21 (14%) patients were able to meet target cell dose of ($2.5 x10^6$ CD34+ cells/kg (median of 4 apheresis). In contrast, 87% of Group II achieved the target dose with a median of 2 aphereses. Initial mobilization protocol was 10 (g/kg of G-CSF alone for both groups. The median WBC count on the day of first apheresis was $21.3x10^3$/(l for Group I versus $39.7x10^3$/(l for Group II and the median CD34+ cell content of the first apheresis yield was 0.08% versus 0.46%, respectively. Nineteen patients in Group I and all Group II completed treatment with a median time to engraftment of AGC>500/(l of 12 and 11 days, respectively and platelet $>20x10^3$/(l of 31 and 13 days, respectively. Outcome analysis revealed that 6/19 patients in Group I died of relapse within less than a year from transplant while only 2/23 of Group II died of the same causes. There were no treatment related deaths in either group. Our results show that definite early predictive features for "hard to mobilize" patients cannot be recognized and that poor mobilization may indicate worse prognosis and outcome for lymphoma patients.

56

PREEMPTIVE THERAPY BASED ON CYTOMEGALOVIRUS ANTIGENEMIA AFTER BONE MARROW TRANSPLANTATION.

Takehiko Mori, Sahoko Matsuoka, Reiko Watanabe, Akaru Ishida, Masatoshi Wakui, Shinichiro Okamoto, Yasuo Ikeda Div. of Hematology, Keio Univ. Sch. of Med., Tokyo, Japan

There have been trials for prevention of cytomegalovirus (CMV) diseases, either using prophylactic or preemptive approach with ganciclovir (GCV), which were not uniformly successful. To treat the patients at highest risk of CMV diseases and minimize the toxicity of GCV, we designed a preemptive therapy based on the degree of CMV antigenemia and acute GvHD. After engraftment, CMV antigenemia was routinely monitored weekly and once it revealed positive, monitored twice a week. If CMV antigenemia at a level of 10 or more positive cells in 2 slides in patients with no or grade I acute GvHD, or any positive cells in patients with Grade II-IV acute GvHD was detected, intravenous GCV was initiated at a dose of 10 mg/kg/day for 14 days. If antigenemia was negative after 14-day therapy, GCV was discontinued. If not, GCV was continued at a dose of 5 mg/kg/day or less until antigenemia revealed negative. Fifty two consecutive CMV-seropositive recipients of allogeneic BMT or recipients of CMV-seropositive allografts were entered into this study and 48 were evaluable. Most of the patients had hematologic malignancies. Twenty three received marrow from HLA-identical siblings and 25 from unrelated donors. Prophylaxis for GvHD was attempted with cyclosporine or tacrolimus with short term methotrexate in most cases. All patients received CMV hyperimmunoglobulin weekly, and HSV-seropositive patients also received intravenous acyclovir (from Day-3 to +14). In 48 evaluable patients, 22 patients (46%) developed CMV antigenemia and 8 out of the 22 patients (45%) developed CMV disease (7; gastroenteritis, 1; retinitis). All 8 patients had Grade II-IV GvHD. In 6 patients, CMV antigenemia became positive after the manifestation of CMV diseases. Incidence of CMV antigenemia/disease was significantly higher in patients with Grade II-IV GvHD than those with Grade 0-I ($P<.05$). CMV pneumonia and CMV-associated deaths were not observed. Neutropenia due to GCV was observed in 17% of patients treated, which was successfully treated with G-CSF. These results suggest that our preemptive approach is quite effective for preventing CMV pneumonia, which is often fatal, but not effective for GI disease and probably retinitis.

57

TRANSPLANTATION-RELATED MORTALITY AND TREATMENT OUTCOME IN NON-HODGKIN'S LYMPHOMA PATIENTS WITH 'POOR' MOBILIZATION OF PERIPHERAL PROGENITOR CELLS.

Sunil A. Reddy, Sandra J. Horning, Karl G. Blume, and Keith E. Stockerl-Goldstein. Stanford University Medical Center

Many groups of investigators have reported that a minimum of 2 x10^6 $CD34^+$ cells/kg are necessary to allow rapid engraftment of white blood cells and platelets following high-dose therapy and autologous peripheral blood progenitor cell (PBPC) support. This analysis was performed to evaluate the outcomes of patients who are 'poor' mobilizers of PBPC's who collect < 2 x 10^6 $CD34^+$ cells/kg versus 'good' mobilizers who collect (2 x 10^6 $CD34^+$ cells/kg in a single apheresis.

Methods. One hundred seventy-two consecutive patients with non-Hodgkin's lymphoma (NHL) were treated with high-dose therapy and autologous hematopoietic cell support between 8/1/94 and 7/31/98. Patients received cyclophosphamide and G-CSF followed by apheresis of PBPC's. The PBPC's were separated on a Percoll gradient and purged using monoclonal antibodies and complement.

Results. The day of last follow-up is 11/30/98, with a minimum follow-up of 132 days and a median follow-up of 2.4 years. Thirty-four patients were 'poor' mobilizers of PBPC's (19.8%) with a median collection of 0.84 x 10^6 $CD34^+$ cells/kg (range 0-1.9 x 10^6 $CD34^+$ cells/kg). One hundred thirty-eight patients were 'good' mobilizers of PBPC's with a median collection of 4.6 x 10^6 $CD34^+$ cells/kg (range 2.0-29.1 x 10^6 $CD34^+$ cells/kg). The only significant predictive factor for 'poor' mobilization is that they are more likely to be women (65%) than men (35%, p=0.003). 'Poor' mobilizers received autografts utilizing purged bone marrow in 15 (44%), purged PBPC's in 14 (41%) and purged bone marrow plus purged PBPC's in 5 (15%). All 'good' mobilizers were autografted utilizing purged PBPC's alone. Engraftment of white blood cells and platelets was significantly slower in 'poor' mobilizers. There was a significantly higher transplant-related mortality (TRM) in the 'poor' mobilizers, 11%, compared with 'good' mobilizers, 3%, p=0.05. Three-year Kaplan-Meier estimates of event-free survival (EFS), overall survival (OS) and relapse (REL) are presented and demonstrate no significant differences. 'Good' mobilizers had a respective EFS, OS, and REL of .47, .59, and .50 as compared to 'poor' mobilizers values of .41, .55, and, .48. The corresponding p values are .60, .37, and .72. Data evaluating predictors of 'poor' mobilization and an economic analysis associated with the costs of 'poor' mobilization will also be presented.

Conclusion. Patients who are 'poor' mobilizers of PBPC's have equivalent EFS, OS and REL following high-dose therapy and autologous hematopoietic support, however, TRM is significantly higher in this group.

58

VALIDATED PREDICTIVE MODEL FOR RELAPSE AFTER HIGH-DOSE CHEMOTHERAPY (HDC) WITH AUTOLOGOUS STEM-CELL SUPPORT (ASCS) FOR HIGH-RISK PRIMARY BREAST CANCER (HRPBC).

Y Nieto, PJ Cagnoni, EJ Shpall, X Xu, RB Jones, SI Bearman. University of Colorado BMT Program

A retrospective analysis of 176 HRPBC pts (defined by 10+, 4-9 involved nodes, or inflammatory breast cancer [IBC]), treated with HDC and ASCS, was performed. All pts received high-dose cyclophosphamide, cisplatin and BCNU (STAMP-I), followed by radiotherapy and tamoxifen, if either ER+ or PR+. These pts had survived the transplant, and were either relapse-free at (2 years of follow-up (F-U), or had relapsed at any time post-HDC. Median F-U is 45 (range 12-74) months. We defined nodal ratio as the quotient (number of (#) positive (+) nodes / # sampled nodes(. An association was found between risk of relapse and nodal ratio (p=0.001), ER(-) (p=0.001), PR(-) (p<0.01), tumor size (p<0.01), stage (p=0.01), tumor grade (p<0.05), and clinical IBC (p<0.05). The following did not correlate with relapse: absolute # of + nodes, pathologic IBC (dermal lymphatic involvement), vascular or lymphatic vessel invasion, multifocality, extensive intraductal component, DNA ploidy, S-phase fraction, lymphatic extranodal extension, size of involved nodes, menopausal status, gender, bilateral synchronous tumors and family history of BC. In a multiple logistic regression analysis, nodal ratio, size and ER/PR negativity (both ER and PR negative) were independent predictors. A scoring system was derived:

Score = (size x 0.15) + (ratio x 3.05) - (ER/PR x 1.19).

In this formula, size is entered in cm and ER/PR is assigned "0" if both negative, and "1" if either one or both are positive. Scores of <2.41 or >2.41 allocate pts to low or high-risk groups, with probabilities of relapse of 15% and 85%, respectively. Differences in relapse-free (RFS) or overall survival (OS) between pts with a high and a low score are highly significant (p<0.000001). The model has these properties: sensitivity/specificity: 60%/90%, positive/negative predictive value: 65%/88%, accuracy: 83%.

The model was validated in an independent sample of 244 HRBC pts treated at Duke University with STAMP-I. The differences in RFS and OS between low- and high-score pts were highly significant (p<0.000001).

This model will be used to select high-score patients for future research.

59

ALLOGENEIC MARROW TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA (SAA) - A TWENTY YEAR PERSPECTIVE.

M. O'Donnell, W. Hu, A. Stein, J. Palmer, M. Amylon, N. Chao, P. Parker, D.S. Snyder, A. Nademanee, E.P. Smith, K. Margolin, R. Spielberger, A. Kashyap, J. Rosenthal, R. Krance, K. Blume and S.J. Forman. City of Hope National Medical Center and Stanford University

A retrospective chart review of 88 patients transplanted from sibling donors for SAA between May 1975 and Dec. 1997, was conducted to assess impact of preparative regimens and GVHD prophylaxis as well as pre-BMT factors such as age, prior transfusion and donor/recipient sex match on engraftment and overall survival. Preparative regimens were Cytoxan (CY) alone 53 pts, CY+ radiation 10 pts and CY + ATG 25 pts. GVHD prophylaxis was MTX and prednisone (PSE) 15 pts, Cyclosporin (CSA)/PSE 33 pts and CSA + MTX (+/- PSE) 37 pts. Median age at BMT was 23 yrs (4-47) and median time to BMT was 25 days (10-3600 d). Six pts (7%) had either initial (2 pts) or late (4 pts) graft failure among 84 pts surviving beyond 14 d; the late graft failures occurred between d 130 and 370 post BMT. All graft failures occurred in pts prepared with CY alone; however, neither preparative regimen (p=0.10) GVHD prophylaxis or any pre BMT pt characteristics predicted for graft failure. Five graft failure pts received ATG and CY or nitrogen mustard for second BMT and all achieved successful engraftment. The incidence of acute GVHD (grade 2-4) has decreased significantly in the last 20 years from 75% in pts receiving MTX/PSE to 8% in pts receiving CSA/MTX in conjuction with CY + ATG for conditioning. The overall incidence of acute GVHD (grade 2-4) was 31%; this did have a significant impact on survival at d 100 (91% for grade 0-1 and 83% for grade 2-4 p = 0.01). Chronic GVHD developed in 38/74 pts (51%) it progressed from acute in 13 pts and appeared de novo in 25 of these pts. Chronic GVHD was limited in 21 pts and extensive in 17 pts. The incidence of chronic GVHD has decreased to 25% in the last five yrs. Overall, survival at 2 yrs has improved dramatically in the last 20 yrs from a 43% in the 1970s, to 78% in the 1980s and 88% currently. No one factor has significantly affected overall survival independently although there are trends suggested by better GVHD prophylaxis (90% 2 yrs OS with CSA/MTX + 1-PSE vs 79% for CSA/PSE and 67% MTX/PSE) and improved conditioning (88% 2 yrs OS with CY/ATG vs 75% for CY alone. Age over 30 yrs was the only pt dependent factor which predicted for a lesser survival at 2 yrs (65% vs 85% for pts less than 30 yrs) (p = 0.01). Allogeneic BMT offers an excellent outcome with good quality of life in young patients with SAA.

60

TREATMENT OF ADVANCED MYELODYSPLASTIC SYNDROME (MDS) WITH A REGIMEN INCLUDING RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) PRECEEDING ALLOGENEIC BONE MARROW TRANSPLANTATION.

Shinichiro Okamoto[1], Satoshi Takahashi[2], Masatoshi Wakui[1], Akaru Ishida[2], Ryuji Tanosaki[1], Yasuo Ikeda[1], and Shigetaka Asano[2] Keio BMT Program, School of Medicine, Keio University[1], The Institute of Medical Science, The University of Tokyo[2], Tokyo, Japan

Treatment of MDS generally has been unsatisfactory, and allogeneic bone marrow transplantation (BMT) is currently the only curative treatment for MDS resulting in reported 19% to 56% disease-free survival (DFS) rates. However, even with an allogeneic BMT, patients with advanced disease morphology (RAEB or RAEB-T) or MDS-related secondary AML (sAML) had a higher relapse rate and a lower DFS rate after transplantation compared with less advanced diseases. To investigate whether the myeloid cell-specific action of G-CSF can be used to accomplish more effective eradication of myeloid leukaemic cells, we have administered G-CSF simultaneously with pretransplantation conditioning consisting of total body irradiation (TBI) and cytosine arabinoside (Ara-C). We have examined the efficacy of this conditioning regimen in 13 patients with RAEB (n=4), RAEB-T (n=1), or sAML (n=8). The patients were between 18 and 53 years old (median, 43). TBI (total dose 12 Gy) was given in 4 to 6 fractions on days -9 and -8 or days -10, -9, and -8. Ara-C was administered i.v. over 2h at a dose of 3 g/m^2 every 12h for 4 consecutive days between days -5 and -2. Recombinant human G-CSF was administered by continuous infusion at a dose of 5 (g/kg/day. Infusion of G-CSF was started 12h before the first dose of Ara-C and stopped at the completion of last dose of Ara-C. All patients received marrow transplantation from HLA-identical sibling donors. Twelve patients received cyclosporine and one patient received tacrolimus in combination with methotrexate (MTX) as prophylaxis against GVHD. Engraftment and complete remission were obtained in all cases. No regimen-related deaths occurred, and no side effects related to the addition of G-CSF were observed except for transient mild bone pain. At a median follow-up time of 39 months, the projected 5-year disease-free survival and 5-year overall survival were 67.7% and 75.5%, respectively, with only one case showing cytogenetic relapse. Projected relapse and nonrelapse mortality rates at 5 years were 8.3% and 26.7%, respectively. This preparative regimen including G-CSF is feasible, and preliminary results seem to be encouraging. However, a larger trial is clearly warranted to evaluate its efficacy.

61

INVESTIGATION OF THREE C-MYC RETROVIRUS-TRANSFORMED MYELOID LEUKEMIA LINES FOR POTENTIAL SHARED TUMOR ANTIGENS IN A GRAFT-VERSUS-LEUKEMIA MODEL.

A. Patterson, M. Hsieh, and R. Korngold. Kimmel Cancer Institute, Thomas Jefferson Medical College, Philadelphia, PA 19107

A *c-myc* retrovirus-transformed myeloid leukemia line, MMB3.19, was constructed to investigate graft-versus-leukemia (GVL) activity in a syngeneic and allogeneic murine bone marrow transplantation (BMT) model. It was previously determined in this model that leukemia-presensitized $CD4^+$-enriched T cells are capable of mediating GVL activity to MMB3.19 challenge while minimizing graft-verus-host disease (GVHD). Two other similarly derived myeloid leukemia lines, MMB1.10 and MMB2.18, were developed to investigate potential shared tumor antigens in this GVL model. Morphologically, all 3 tumor lines are blastic with a high cytoplasmic to nuclear ratio. MMB2.18 demonstrates many dendritic processes while MMB 1.10 and 3.19 are more rounded. Flow cytometric analysis of the 3 leukemia lines revealed surface molecule expression of Mac-1, Mac-2, F4/80, LFA-1, B7-2, H-$2K^b$, macrophage scavenger receptor, and B7-1 (only MMB2.18 and 3.19). *In vitro* cross-proliferation studies in which MMB3.19-primed splenocytes were restimulated with either MMB1.10, 2.18, or 3.19 demonstrated that splenocytes proliferated upon restimulation with MMB1.10 or 3.19, but not MMB2.18. Furthermore, survival GVL assays using donor MMB3.19-primed $CD4^+$ T cells or unfractionated T cells and subsequent leukemic challenge with either MMB1.10, 2.18, or 3.19 showed increased survival in groups receiving MMB1.10 or 3.19, but not 2.18. These data suggest that MMB1.10 and 3.19 may express a common tumor antigen(s) while MMB2.18 lacks such cross-protective characteristics.

62

RAPID AND DURABLE ENGRAFTMENT IN TWO PATIENTS WITH HIGH RISK CML USING UNEXPANDED AND EXPANDED UMBILICAL CORD BLOOD STEM CELLS.

A.L. Pecora, P. Stiff A. Jennis, S. Goldberg, R. Rosenbluth, P. Price, J. Douville, R. Armstrong, A. Smith and R. Preti. Hackensack University Medical Center, Hackensack, NJ; Loyola University Medical Center, Maywood, IL; and Aastrom Biosciences, Ann Arbor, MI

Unrelated donor cord blood transplants in patients with a diagnosis of CML and those receiving a low nucleated cell dose, in the HLA mismatched setting, and in older patients, are associated with a greater likelihood of delayed engraftment, graft failure, and non-relapse transplant related events, respectively. In an attempt to improve on these outcomes we treated two patients with CML; a 48 year old (107 kg) male (pt.1) in early recurrent blast crisis and a 56 year old (72 kg) female (pt. 2) in accelerated phase, with fractionated TBI (1200 cGy), ATG (90 mg/kg) and cyclophosphamide (120 mg/m^2), using unexpanded and ex-vivo expanded unrelated cord blood. The cord blood units were thawed and divided with approximately 90% of the unexpanded unit infused on day 0 (pt. 1: 6/6 match; $1.1x10^7$/kg) (pt. 2: 5/6 match; $1.0x10^7$/kg). The remaining cells (pt. 1: $1.5x10^8$; pt. 2: $1.3x10^8$) were expanded in AastromReplicell perfusion bioreactors for 12 days, perfused with media containing PIXY, Flt3, and erythropoietin and then infused (pt. 1: $3.4x10^8$; pt. 2: $2.3x10^8$). Prophylaxis for infection and GVHD included oral ciprofloxacin, low dose amphotericin, cyclosporine and prednisone. Patient one engrafted rapidly (neutrophils 500 and 1000 @ day 31 and 33: platelets 20, 50 and 100 @ day 52, 92, 147), had no significant adverse transplant related events except for transient CHF, no GVHD and remains in a cytogenetic remission (100% donor) @ 13months. Patient two also engrafted rapidly (neutrophils 500 and 1000 @ day 25 and 34: platelets 20 @ day 60), had no significant transplant related events (never required i.v. antibiotics), no GVHD, and remains in a cytogenetic remission (100% donor) @ day 80. In conclusion, ex-vivo expanded cord blood stem cells appear to promote hematopoietic engraftment and reduce adverse transplant related events in high risk unrelated cord blood transplant recipients, warranting further investigation.

63

ABSOLUTE NUMBER OF CD 34 + CELLS IN PERIPHERAL BLOOD PREDICTS SUCCESSFUL APHERESIS COLLECTION OF PROGENITOR CELLS IN INFANTS AND CHILDREN.

S. Kelly, C. Rollo, K. Prothe, R. Quinones, N. Foreman, R. Giller. The Children's Hospital / University of Colorado Health Sciences Center, Denver

Peripheral blood progenitor cell harvests in pediatric oncology patients sometimes result in products which contain suboptimal numbers of CD34+ cells. This necessitates repeat procedures in order to achieve a dose of progenitor cells sufficient to rescue hematopoietic function. Therefore, we undertook a study to determine if the absolute number of CD34+ cells in peripheral blood predicted the yield of CD34+ cells in the apheresis product. Efficient collection was defined as a yield of (1.0e6 CD34+ cells/kg per apheresis. Data was compiled from 42 apheresis procedures in 14 patients over a period of one year and analyzed using standard regression analysis. CD34+ content of peripheral blood and apheresis product was determined by flow cytometric analysis using the ISHAGE technique. Absolute CD34+ cells/ul was defined as WBC multiplied by percent CD34+ events.

Our analysis showed that 20 out of 24 harvests with an absolute CD34+ count <10/ul resulted in a product CD34+ dose < 1.0e6/kg. Regression analysis did not show linear correlation between CD34+ content in peripheral blood and content in the final product for low absolute CD34+ counts (<10/ul). In contrast, at an absolute CD34+ count (10/ul, only 3 out of 18 harvests failed to yield a dose of (1.0e6/kg. Moreover, regression analysis showed correlation between absolute CD34+ count and product yield for absolute CD34+ counts (10/ul. The ability of peripheral blood absolute CD34+ to predict apheresis yield was statistically significant (chi square; $p<0.01$). These results suggest that absolute peripheral blood CD34+ count can be used to determine the optimal time to collect progenitor cells in pediatric oncology patients.

64

TACROLIMUS VERSUS CYCLOSPORINE IMMUNOSUPPRESSION: RESULTS IN ADVANCED STAGE DISEASE PATIENTS WITH COMPARISON TO HISTORICAL CONTROLS

D. Przepiorka[1], M. Horowitz[2], P. Bartels[3], D. Buell[3], MJ. Zhang[2], W. Fitzsimmons[3], J. Erdman[3], C. Huang[3], E. Hodosh[3], R. Maher[3], J. Wingard[4] [1]Texas Children's Hospital, Houston, TX. [2]IBMTR, Milwaukee, WI. [3]Fujisawa Healthcare, Inc., Deerfield, IL. [4]U of Florida, Gainesville, FL

A Phase III comparative trial of tacrolimus- versus cyclosporine-based graft-versus-host disease (GVHD) prophylaxis for HLA-identical sibling bone marrow transplantation showed less GVHD but poorer survival in the tacrolimus arm. However, a significantly greater proportion of patients with advanced stage disease were randomized to tacrolimus-based therapy. To further evaluate the comparability of treatment arms in this study, the International Bone Marrow Transplant Registry (IBMTR) database was used to select matched controls for patients with advanced disease in the two arms of the randomized trial. All controls received cyclosporine-methotrexate therapy at a North American center between 1990 and 1995 following receipt of bone marrow from an HLA-identical sibling donor. Two controls were identified for each of 100 of the 116 advanced disease patients in the Phase III trial. Controls were matched for age (within 5 years), disease, and pre-transplant disease status.

Consistent with the study results, the Kaplan-Meier estimate of Grade II-IV acute GVHD was significantly lower ($p<0.01$) in tacrolimus-treated clinical trial patients compared with their matched IBMTR controls (28% versus 50%, respectively). Two-year survival for tacrolimus-treated clinical trial patients was similar to their matched controls (27% versus 24%, respectively). Both Grade II-IV acute GVHD and 2-year survival of the cyclosporine-treated clinical trial patients were similar to their matched controls (acute GVHD: 58% versus 45%, respectively; survival: 42% versus 45%, respectively). However, the IBMTR controls matched to the tacrolimus group had significantly poorer ($p<0.01$) 2-year survival than the IBMTR controls matched to the cyclosporine group (24% versus 45%, respectively). These results support the hypothesis that the survival difference in the Phase III trial resulted from an imbalance in the underlying risk factors for death in the two groups rather than the randomized immunosuppressive regimen.

65

THE EFFECT OF SEQUENTIAL EX VIVO CYTOKINE EXPOSURE ON THE GENERATION OF DENDRITIC CELLS FROM MOBILIZED CD34+ BLOOD PROGENITOR CELLS.

Roman-Unfer S, Potter T, Quinones R, McNeice IK, Smith M, Steele A, Stoltz J, Cagnoni PJ, Bearman SI, Jones RB, Nieto Y, Shpall EJ. U of Colorado, Denver, CO

Dendritic cells are professional antigen-presenting cells that play a crucial role in the immune response. Previous studies using dendritic cells generated from peripheral blood mononuclear cells (PBMCs) have revealed two functionally distinct subsets of DCs (immature vs. mature) that differ in both function and phenotype. Immature DCs are typically generated in the presence of GM-CSF and IL-4 and are efficient in antigen uptake and processing. Following exposure to TNF-(, these cells mature and have an increased allo-MLR, as well as a decreased ability to take up and process antigen. In this study, PBPCs were collected from patients following mobilization with granulocyte stimulating factor (G-CSF). The $CD34^+$ cells were immunomagnetically isolated from a single apheresis product with the Baxter Isolex 300i device. These $CD34^+$ cells were resuspended in X-vivo 15 medium containing GM-CSF (50 ng/ml) and SCF (20 ng/ml) at a final cell concentration of 1.0 x 10^6 cells/ml, and then incubated at 37(C in 25 cm^2 tissue culture flasks (Costar; Cambridge, MA) for two weeks. TNF-((10 ng/ml), was then added and the cells were incubated for an additional week. This method resulted in product at week two where none of the cells exhibited typical DC morphology on cytospin and expressed the following markers upon flow cytometric analysis: CD11c (40-50%), HLA-DR(7-13%), CD40(1-2%), and CD4 (0.1-0.2%). Functionally these cells did not exhibit enhanced allo-MLRs. Following the exposure of these cells to TNF-(for one week, 30% of these cells exhibited typical DC morphology on cytospin and revealed enhanced expression of HLA-DR (31%), CD11c (60-70%), CD40 (13-34%), and CD4 (7-23%) upon flow cytometric analysis. In addition these cells were capable of eliciting a potent allogeneic mixed lymphocyte response, with stimulation indices greater than 100-fold when used at a ratio of 1 DC per 100 peripheral blood lymphocytes (PBL). Results of antigen uptake studies will be presented.

66

EFFECT OF MOLECULAR TYPING OF HLA CLASS I ANTIGENS ON SEVERE ACUTE GVHD FOLLOWING UNRELATED BMT.

Rugo HS, Sayre P, Capper J, Damon L, Ries C, Linker C. University of California, San Francisco

We performed DNA typing on class I antigens from patient and donor after unrelated BMT complicated by severe early acute GVHD to assess the impact of mismatching. A 49 y.o. male with CML underwent matched unrelated donor BMT. Diagnosis of CML was made 8 months prior to BMT; a 5 month trial of interferon was poorly tolerated. Patient and donor were typed for class I, A and B loci, by standard lymphocytoxicity using local and commercial typing trays. The C locus was typed using a commercial sequence specific priming (SPP) kit. Class II alleles were typed by SSP using locally produced reagents. Typing of the patient:

A3A30B7B44CW*0501CW*0702DRB1*1501DRB1*1301DQB1*06DRB3*030 1DRB5*0101 and donor:

A3A30B7B44CW*07 CW*0702DRB1*1501DRB1*1301DQB1*06DRB3*0301DRB5*0101 demonstrated a single mismatch at C locus allele. The donor was female, 35 y.o., G1P1 and A+. The patient was O+; both patient and donor were CMV negative. Marrow was depleted of red cells using the Cobe Spectra cell separator. Following 1200 cGy TBI and 120 mg/kg cyclophosphamide, 0.38 x 10^8 mononuclear cells/kg were infused into the patient. GVHD prophylaxis consisted of cyclosporine, methotrexate, and steroids. The patient initially did well with neutrophil engraftment (ANC>1000/ul) on day 23. On day 26 a diffuse rash consistent with acute GVHD developed. Despite aggressive treatment with high dose steroids, tacrolimus and Cellcept, grade IV GVHD involving skin, GI tract and liver developed and progressed. The patient expired on day 46. Retrospectively, patient and donor were sequenced for the A and B loci using automated four color fluorescent sequencing and dye terminator chemistry. Exons 2, 3 and 4 of the A locus and exons 2 and 3 of the B locus were sequenced. Results of the patient followed by donor are shown below:

A*0301	A*3001	B*0702	B*4402
A*0301	A*3004	B*0702	B*4403

These results demonstrate additional mismatching at one A and one B locus as well the known mismatch at the C locus. This patient and donor were a three antigen mismatch for class I alleles, likely resulting in the severe acute GVHD observed. DNA typing of class I antigens should be performed on prospective donors before unrelated donor BMT for CML, especially when antigens with multiple alleles (such as A30 and B44) are found on serologic typing and when other disparities (such as C mismatch) are known.

67

VISUALIZING THE KINETICS OF TUMOR CELL CLEARANCE IN LIVING ANIMALS.

T.J Sweeney, V. Mailander, A.A Tucker, A.B.Olomu, C.H. Contag, R.S. Negrin, Department of Medicine and Department of Pediatrics, Stanford University School of Medicine, Stanford, California

Our laboratories have recently developed a non-invasive method for monitoring tumor progression in the living animal. Tumor cells were tagged with the bioluminescent reporter gene luciferase and engrafted into irradiated mice with severe combined immunodeficiency (SCID). Growth characteristics of the tumor could be followed by the use of an intensified charge coupled device (ICCD) camera. Bioluminescence, detected externally, was proportional to cell number over 4 orders of magnitude. We used this methodology to study the in vivo effects of a novel immunotherapy and standard chemotherapy against a luciferase tagged human cervical cancer cell line (HeLa). These bioluminescent target cells were injected intraparitoneally (ip) into 7 groups of 7 irradiated SCID mice each. 3 groups of mice were treated with a population of human T cells expanded ex vivo, termed cytokine induced killer (CIK) cells. The cells have potent anti-tumor activity against a variety of human lymphoma cell lines both in culture and engrafted into SCID mice. These cells are derived from T cell precursors and co-express the T cell marker CD3 and NK marker CD56. Animals were treated with either a 100:1 E:T ratio on days 1 and 7, a 1000:1 E:T ratio on day 1 alone, or 1000:1 E:T ratio on days 1 and 7. 3 of the groups were treated with chemotherapy, either cyclophosphamide, 5-fluorouracil or cisplatin. The last group received PBS as a control. Mice were imaged weekly for 4 weeks and tumor growth was measured by following the relative intensity of the light emitted from the tumors. All the mice treated with PBS showed a graded increase in light with time. The mice treated with CIK cells revealed attenuated tumor growth in all three groups with several mice in the 1000:1 E:T ratio groups demonstrating complete clearance of tumor signal throughout the 4 week experimental period. The mice treated with 5-fluorouracil and cisplatin demonstrated a reduction of light intensity compared to control animals. In contrast, cyclophosphamide had little in vivo activity. PCR analyses of long term survivors in the cisplatin treated group failed to demonstrate residual tumor. This novel, noninvasive system allowed sensitive, real time spatiotemporal analyses of neoplastic cell growth and facilitated rapid optimization of effective treatment regimens.

68

THE QUALITY OF STEM CELL (SC) COLLECTION PREDICTS FOR MORE RAPID ENGRAFTMENT FOLLOWING STEM CELL TRANSPLANTATION (SCT).

J.R. Schriber, J.C. Alvarnas, D Boudreau, E Simpson, A. Dagis, S.J. Forman, I. Sniecinski. City of Hope and City of Hope Samaritan BMT Program. Duarte CA, and Phoenix AZ.

SCT is increasingly used to treat malignant disease. Typical collection goals are a minimum CD 34 cell count of 2-3.0 x 10^6/Kg, although some studies have suggested that collecting higher CD 34 numbers may hasten platelet count recovery. Little information is available on the "quality" of SC collections. To investigate this, we examined patients (pts) who underwent apheresis with a collection goal of 4-5 x 10^6/Kg CD 34 cells to determine whether pts who achieved this goal in (2 apheresis procedures (group 1) engrafted faster than pts who required more than 2 apheresis (group 2). Fifty five pts have undergone apheresis and subsequent SCT over the past 18 months. Twenty three of these met their SC goal in (2 collections while 32 required more than 2 collections. Pt diagnosis were equivalent and their was no difference in the transplant regiments between the 2 groups. The median number of CD34 cells was significantly higher in group 1. Neutrophil and platelet engraftment were significantly faster in group 1. It is unclear whether this difference is attributable to a difference in quality or whether the higher number of CD 34 cells infused into patients in group 1 accounts for the more rapid engraftment. Previous studies have shown no difference in engraftment beyond a threshold dose suggesting that quality in addition to quantity of cells collected is important in achieving rapid engraftment. Further study on why some patients mobilize rapidly appears warranted.

	GROUP 1 (≤ 2 APHERESIS) (N=23)	GROUP 2 (N=32)	P VALUE
Diagnosis			
Breast Ca	**15**	**21**	
NHL	**5**	**3**	
HD	**1**	**4**	
Other	**2**	**4**	
Collection # (median)	**2**	**4**	
CD 34 #/kg (median x 10^6/kg)	**8.1 (3.6-28.3)**	**4.6 (2.2-29.6)**	**0.003**
Engraftment (days)			
ANC > 500	**9(6-10)**	**10(7-16)**	**0.003**
Plt > 20	**10(9-20)**	**12(9-40)**	**0.002**

69

PROSPECTIVE NEUROCOGNITIVE ASSESSMENT FOLLOWING BONE MARROW TRANSPLANTATION: PART II. INFANTS.

Shimoda, KC, McCabe, MA, Schuman, WB, Dinndorf, PA Children's National Medical Center and George Washington University School of Medicine and Health Sciences, Washington, DC

Bone marrow transplantation (BMT) includes preparative regimens which may be toxic to the developing central nervous system. Infants and children under the age of three may be at particular risk for neurotoxic effects of preparative regimens due to the vulnerable nature of the nervous system in early development. Longitudinal data are presented for 41 children under the age of 3 who underwent BMT for treatment of leukemia or other blood disorders. Patients have been followed prospectively with developmental and/or cognitive testing (Bayley, Bayley-II, McCarthy Scales) at baseline, 6-months post-BMT, and 18-months post-BMT. Overall, young children's developmental levels remain within normal limits at 6-months post-BMT, with significant declines in developmental level demonstrated by 18-months post-BMT. Ongoing evaluation of these patients includes follow-up assessments at 36 months and 60 months post-BMT.

70

HIGHER DOSE G-CSF CAN SALVAGE PATIENTS (PTS) WHO FAIL TO MOBILIZE PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) WITH STANDARD DOSE G-CSF.

I. Sniecinski, J. Schriber, J.Alvarnas, G. Somlo, S. Forman, City of Hope and City of Hope-Samaritan BMT Program, Duarte, CA and Phoenix, AZ

Fifteen to 20% of pts mobilize poorly following standard G-CSF dose (10 (g/kg/day). In this study we evaluated whether higher doses of G-CSF may salvage these poor mobilizers. Between Jan. and Oct. 1998, 50 pts failed to collect adequate CD34+ cells in response to a standard G-CSF dose. PBPC collection began on Day 5 of G-CSF(10 (g/kg/day). CD34+ cells were measured in PBPC product using the ISHAGE protocol. Pts with insufficient CD34+ cell yields (<1x10^6/kg) after the first 3 collections, received augmented doses of G-CSF (10 (g/kg/BID) until sufficient CD34 cells were harvested. Twenty-nine were males and 21 females. The median age was 34 (range 11-56). Diagnoses were: NHL (14), MM (11), HD (8), Breast Carcinoma (8), Acute Leukemia (4), other (4). Table 1 summarizes WBC and CD34 cell yields in PBPC concentrate.

PBPC Concentrate Increase	Before G-CSF Parameters	After G-CSF Increase	Fold Increase
WBC(x10^9)	13.3 (0.8-52)	19.2 (6.2-55)	1.4
CD34(%)	0.10 (0.01-0.20)	0.33 (0.06-1.23)	3.3
CD34x10^6/kg	0.21 (0.01-0.69)	0.82 (0.09-3.15)	3.8

Forty-eight (96%) pts had an increase in CD34+ cells after G-CSF dose escalation. Median increase in WBC yield per apheresis was 1.4 fold. Median increase in the % and total CD34+ were 3.3 and 3.9 fold, respectively. Following G-CSF dose increase, a median 6 (2-16) additional apheresis were required to obtain the target CD34 cell dose for transplantation. Side effects from G-CSF dose escalation included bone pain and headache. No grade 4 toxicity was noted. We conclude that G-CSF dose escalation in pts who mobilize poorly with standard dose G-CSF is an effective strategy for mobilization of CD34+ cells.

71

PROGENIPOIETIN, A POTENT HEMTOPOIETIC STEM CELL MOBILIZING AGENT.

P.R. Streeter, J.M. Mulcahy, J.P. McKearn, W.H. Fleming*. Division of Hematology and Medical Oncology, Oregon Health Sciences University, Portland, OR, USA*, and G.D. Searle, A Monsanto Co., St. Louis, MO, USA*

Progenipoietin (ProGP) is a dual receptor agonist which activates both the flt-3 and the G-CSF receptors. We evaluated the capacity of ProGP to mobilize transplantable hematopoietic stem cells (HSC) and progenitor cells into the peripheral blood (PB) of normal mice. To study the functional activity of circulating HSC, the potential of unfractionated PB to protect lethally irradiated (1100 cGy) congenic recipients was determined. Donor C57B1/6 mice were injected daily for 5 days with either ProGP or PBS/BSA. As little as 5 uL of unfractionated PB from ProGP treated donors protected 100% of lethally irradiated recipients and 1 uL of PB rescued 80% of recipients. In contrast, 100 uL of PB from PBS/BSA treated donors failed to protect irradiated recipients. The PB of all radioprotected recipients of donor cells from ProGP treated animals showed donor-derived (Ly5.2) multilineage hematopoietic reconstitution. In serial transplantation studies, bone marrow from radioprotected chimeric recipients of ProGP treated donors was harvested and then transplanted into lethally irradiated congenic recipient mice (Ly5.1). These secondary recipients demonstrated donor-derived B220+ cells, CD3+ cells and Mac-1+/Gr-1+ cells in PB indicating the engraftment of functional HSC. The engraftment potential of ProGP PB HSC was also confirmed using a competitive repopulation assay. These results indicate that the administration of ProGP leads to the mobilization of substantial numbers of transplantable HSC in normal mice.

72

USE OF S-59 UVA TREATED LEUKOCYTES FOR ALLOGENEIC CELLULAR IMMUNE THERAPY (ACIT):

Sohel Talib, Aaron Reames, Weiqun Liu, Julia McLaughlin and John E Hearst. Cerus Corporation, Concord, CA. and Department of Chemistry, University of California, Berkeley, CA

The proliferation of donor T cells in vivo is the principal cause of GVHD during allogeneic stem transplantation (Allo-SCT).

New strategies are needed to control T-cell alloreactivities in order to make allogeneic transplantation safer and more effective. This study was carried out to test the hypothesis that treatment of human leukocytes in vitro with S-59 and UVA can modulate the T cell proliferation without the loss of their immunological activities. Thus, human leukocytes when treated with varying doses of S-59 + UVA and then activated by either the mixed lymphocytes reaction (MLR) or by anti-CD3 stimulation showed dose dependent inhibition in lymphocyte proliferation. At optimum dose (15 nM and 3 J/cm^2) where proliferation of lymphocytes was inhibited >95% cytokine synthesis (IL-2 and (-IFN) and cell surface antigen expression (CD25 and CD69) remained uneffected. In addition, cytotoxic T cell activity (CTL) of the photochemically treated cell population is retained. Thus the density of S-59 adducts in the genome at the most effective addition doses are sufficient to inhibit DNA synthesis, and yet infrequent enough to allow for the transcription of most genes, allowing for the induction of activated gene expression. Comparison of the concentration dependence of S-59 photoaddition with other psoralens [aminomethytrimethylpsoralen (AMT) and 8-methoxypsoralen (8-MOP)] established that S-59 photoaddition is unique in its degree of specificity for DNA modification, thus reducing other forms of non-specific damage associated with all photochemical processes. Detailed measurements of addition frequencies in lymphocyte genomic DNA were measured by use of radiolabelled psoralens and by an assay based on the inhibition of PCR by the photoadducts. The density range of adducts promoting the desirable T-cell functions was determined to be 1 to 10 per million base pairs. In conclusion, S-59/UVA photochemical treatment of leukocytes provides a novel means of controlling the T-cell alloreactivity during allogeneic stem cell transplantation.

73

CHANGING TREND OF PAEDIATRIC BMT IN SINGAPORE

A.M. Tan[2], P. Tan[1], M.Y. Chan[2]. W. Hwang[1]. Dept. of Haematology[1], Singapore General Hospital and Division of Haematology/Oncology[2], Dept. of Paediatrics, KK Women's & Children's Hospital

Between 7/1985 and 9/1998, 28 children between age of 2 to 12.5 years, mean age 8.1 years, underwent auto/allogenic HSCT for various diseases (ALL=10, AML=5, CGL=4, SAA=4, Thal=3, Fanconi=1, NHL=1). The source of the stem cell was BM=24 and PBSC=4. Types of HSCT were Auto=3, Allo/sib=18 (64%) and MUD=7 (25 %). Conditioning regimen varies, ALL and lymphoma had CY/TBI(7) or BuCy(4), AML had Cy/Mel(2) or Bu/Cy(3), SAA and Fanconi had Cy/ATG (TNI(5), Thal had Bu/Cy(3). MNC cell dose ranged from 1.62 to 30.1x10(8)/kg (mean 5.76x10(8)/kg). GVHD prophylaxis regimen consisted of cyclosporin A & Methotrexate. Neutrophil engraftment was successful in 25/27 patients (92%), range 9 to 44 days. Nonengraftment occured in 2/27 patients (17%), in 1 SAA & 1 Thal. 1 patient died of mycosis at Day 10 and before engraftment and was not evaluated. Overall survival 12/28=43% (Day from BMT ranged 55 to 1585 mean=766 days). Of the 16/28 patients died (57%), 6/16 died from transplant related complications and 3/6 were MUD transplant, the other 10/16 died from rejection/relapsed of the illness. 76% (19/25) allogenic transplant had AGVHD, only 3 had severe GVHD grade 3 or > and all these 3 died from complication of GVHD or Rx or disease relapse. Chronic GVHD occured in 6/25 patients (24%).

Prior to 1994, supportive care was not optimal and patients were in poor clinical conditions with active leukemia or infection before transplant and hence all 9 cases of HSCT (2 auto, 7 allo) died. 1 died from transplant related complication and 8 died from relapse/rejection. Since 1994 to early 1998, a specialised adult BMT team, with input from paediatricians, performed 17 HSCT; 7MUD & 10 allogenic transplant. 10 survivals (59%) ranged from day 291 to 1585 (mean days of 927). Of 7 died, 5 had transplant related complications and 2 had disease relapse. (4 of these were MUD transplants). Since early 1998, 4 HSCT were performed in the new Children's Hospital 2 of which were too early to analyse.

74

GM-CSF BASED TUMOR CELL VACCINES ELICIT POTENT ANTITUMOR IMMUNITY AFTER ALLOGENEIC TCD BMT.

Takanori Teshima, Nicolas Mach**, Yani S. Brinson*, Geoffrey R. Hill*, Glenn Dranoff**, and James L.M. Ferrara#. Departments of *Pediatric Oncology and **Adult Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, #Departments of Internal Medicine and Pediatrics, University of Michigan Cancer Center, Ann Arbor, MI*

Immunotherapy offers an attractive approach to eradicating minimal residual disease (MRD) after BMT, and we have explored an immunization strategy using irradiated B16 melanoma cells (H-2^b) that were engineered to secrete GM-CSF (B16-GM vaccine) to induce specific immunity to wild type B16. Allogeneic BMT was performed using donor and recipient strains that differed by either minor and MHC antigens (SJL((B6SJLF1) or minor antigens alone (LP((B6). Recipients were given 5 x 10^6 donor bone marrow and 1-2 x 10^6 donor T cells following 1100cGy TBI. Syngeneic and T cell-depleted (TCD) donors were used as non-GVHD controls. B16-GM vaccine was injected sc.6 weeks after BMT and animals were subsequently challenged with wild type B16 2-4 weeks later. All unvaccinated animals showed 0% tumor free survival (TFS) by 3 weeks after tumor challenge and their splenocytes did not respond to B16 stimulators in vitro. B16-GM vaccine provided potent antitumor immunity after TCD BMT (77% TFS) and it was at least as powerful as that observed after syngeneic BMT (54% TFS). In vitro analysis of splenocytes one week after vaccination revealed marked proliferation and cytokine production (GM-CSF, IFN-(, IL-2, IL-4, IL-5, IL-10) to B16 cells in vitro. In contrast, vaccination was less protective in recipients given allogeneic T cells with mild GVHD (27% TFS) and afford no protection in animals with moderate GVHD (0% TFS). Thus, the magnitude of antitumor immunity was inversely correlated with the severity of GVHD, suggesting that GVHD-associated immunosuppression limits the protective effect of vaccinations after allogeneic BMT. This result demonstrates that immunologic reconstitution after TCD BMT can provide significant protection of the immunosuppression if GVHD is prevented. This antitumor immunity was long-lasting since 100% (4/4) of the TCD recipients survived rechallenge with wild type tumor 5 months after vaccination. Surprisingly, vaccination did not exacerbate GVHD in either BMT model. These data suggest that the combined approach of TCD (to prevent GVHD) followed by GM-CSF vaccination (to eradicate MRD) may offer a novel strategy to improve disease free survival in patients receiving allogeneic BMT for malignancy.

75

ANTIEMETIC EFFICACY OF THREE SERONTONIN ANTAGONISTS (SA) DURING HIGH-DOSE THERAPY (HDT) AND AUTOLOGOUS STEM CELLS TRANSPLANTATION (ASCT), A PROSPECTIVE RANDOMIZED STUDY.

J Slaby, M Tmeny, B Prochazka, P Klener. 1st Department of Medicine, General University Hospital of Charles University, Prague, CR

The aim of the study: To compare the antiemetic efficacy of three parenteral SA during conditioning regimen prior to ASCT.

Patients and methods: 45 malignant lymphoma patients (median age 38 y M:F 30:15) undergoing regimen BEAM and ASCT, were randomized for granisetron 3mg once a day (G), tropisetron 5mg once a day (T) or ondansetron 8mg twice a day (O) administration. The groups were comparable as to age, sex and experience of previous nausea and/or vomitus. The patients recorded the duration of nausea and numbers of vomitus for 10 d. *Failure of antiemetic therapy* was defined as a nausea $\geq$ 4 hours and/or $\geq$ 3 vomitus/24 h and *failure of emesis control* $\geq$ 3 vomitus/24 h. Both the period of chemotherapy (6 days) and the follow-up period (10 days) were evaluated.

Results: The antiemetic therapy response (no failure of therapy) was observed in 76% and 49%, the emesis control was attained in 98% and 73% of patients during the chemotherapy and follow-up periods, respectively. The efficacy of three SA was comparable during the chemotherapy period. However, the antiemetic therapy response and emesis control were significantly better in the granisetron and in tropisetron groups compared to the ondansetron group during follow-up period (10 d).

TABLE: GTOP

failure of antimet. Th -	**6 d**	**5(33%)**	**2(13%)**	**4(27%)**	**0.40**
failure of antiemet. Th -	**10d**	**7(47%)**	**5(33%)**	**12(80%)**	**0.03**
failure of emesis contr. -	**10 d**	**4(27%)**	**1(7%)**	**7(47%)**	**0.04**

Conclusion: All three SA have identical efficacy during high-dose chemotherapy (BEAM) administration. Tropisetron and granisetron however, provide better antiemetic control compared to ondansetron during postchemotherapy period, when factors other than chemotherapy along can influence emesis and nausea induction.

76

EFFECT OF PHOTOCHEMICAL TREATMENT WITH S-59 PSORALEN AND UVA LIGHT ON GVH AND GVL REACTIVITY OF ALLOGENEIC MURINE T-CELLS.

Robert L. Truitt, Bryon D. Johnson, Sohel Talib and John E. Hearst. Department of Pediatrics and Cancer Center, Medical College of Wisconsin, Milwaukee, WI, Cerus Corporation, Concord, CA, and Department of Chemistry, University of California, Berkeley, CA

The purpose of this study was to analyze the effects of photochemical treatment ex vivo with S-59 (a psoralen) and UVA light (S-59 PCT) on graft-vs-host (GVH) and graft-vs-leukemia (GVL) reactivity in murine models of MHC-mismatched and presensitized MHC-matched BMT. S-59 PCT limits the proliferation of donor T-cells by intercalating and crosslinking DNA. The effect of S-59 PCT on T-cells was dependent upon the concentration of S-59 and the UVA exposure used (3 to 30 J-nM/cm^2). At certain doses, activated S-59 PCT T-cells secreted cytokines (IL2, (IFN, IL10) and expressed activation markers (e.g., CD25 and CD69), but showed limited proliferation in vitro. Using an MHC-mismatched model of T-cell depleted BMT (C57BL/6 into AKR; $H2^b$ into $H2^k$), we found that the incidence and intensity of GVH reactivity of PCT-treated cells was S-59, UVA, and T-cell-dose-dependent. Addition of S-59 PCT T-cells to TCD MHC-mismatched BM resulted in elimination of residual host cells, conversion to complete and stable donor chimerism in T, B and monocytic lineages, and improved survival rates. Chimeras given S-59 PCT T-cells resisted a challenge of AKR acute T-cell leukemia cells, indicating that GVL reactivity was not lost. In the absence of the S-59 PCT T-cells (T-depleted BM only), higher rates of mixed chimerism, graft failure and leukemia-related deaths were observed. Lethal GVHD developed in control mice given untreated T-cells or cells treated with S-59 or UVA alone. To assess the effect of S-59 PCT on presensitized donor cells, an MHC-matched model (B10.BR into AKR; $H2^k$ into $H2^k$) was used. Presensitization of MHC-matched donors to host alloantigens (by multiple i.p. injections of host splenocytes) induced hyperacute GVH reactivity, and more intense S-59 PCT treatment was required to prevent or diminish GVH reactivity. With some treatment regimens, S-59 PCT cells retained GVL reactivity and showed significantly reduced GVH reactivity. When S-59 PCT treatment was sufficient to eliminate GVH reactivity of presensitized donor T-cells, the GVL effect also was eliminated. These results indicate that S-59 PCT treatment is a potential approach to controlling T-cell alloreactivity in vivo without loss of their beneficial activities, but that the therapeutic window in mice is narrow.

77

FLUDARABINE-BASED CONDITIONING FOR ALLOGENEIC TRANSPLANTATION IN A 40 YEAR OLD DIALYSIS PATIENT WITH SICKLE CELL DISEASE.

Koen van Besien M.D, Amelia Bartholomew M.D., Wendy Stock M.D, David Peace M.D, Steve Devine M.D, Dori Scherr M.S., Jeff Sossman M.D and Ronald Hoffman M.D- University of Illinois at Chicago, Chicago, Illinois, 60612

Background. Although allogeneic transplantation can be curative for patients with sickle cell disease, the toxicity of conditioning regimens has precluded its use in adults with significant end-organ damage. Newer conditioning regimens have been developed that are considerably less toxic and that may broaden the applicability of allogeneic transplantation in this disorder.

Case report. We report the case of a 40 year old woman with sickle cell disease and extensive end-organ damage. She had suffered numerous pain crises, had been extensively transfused, had developed multiple red blood cell antibodies and was suffering from end-stage renal failure. This patient underwent an allogeneic stem cell transplantation from her HLA-identical sibling after conditioning with fludarabine, ATG and melphalan. Engraftment occurred promptly. More than 9 months after transplantation, the patient continues to be free of sickle cell disease and in good condition.

Conclusion The use of conditioning regimens with limited toxicity allows the use of allogeneic transplantation in adults who have end-organ damage and constitutes a curative treatment for highly symptomatic patients.

78

PARMACOKINETICS OF INTRAVENOUS BUSULFAN IN HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT).

W.P Vaughan, P. Cagnoni, H. Fernandez, W. Hu, A. Kashyap, V. Gain, J. Wingard, S. Tarantolo, B.S. Andersson. U of AL at Birmingham, U of CO, U of Miami, Stanford U, City of Hope, U of FI, MD Anderson Cancer Center

Oral busulfan (BU) is a well-established ablative component of many hematopoietic stem cell conditioning regimens. However, many studies have demonstrated wide pharmacokinetic variability. BU levels can be an important determinant of regimen-related toxicities or graft rejection. An IV form of BU (Busulfex™) has been developed which reduces the variability of BU AUC. Herein, we will report the pharmacokinetic data of 103 patients that participated in one of two Phase II open-label, multi-center trials using Busulfex (0.8 mg/kg Q6h x 16) and cyclophosphamide (60 mg/kg/d x 2) as the preparative regimen for HSCT (42 auto and 61 allo). Sixty-three patients were male and 43 were female. Transplants were performed for acute leukemia (33), CML (17), lymphoma (45), and MDS (9). The patients ranged in age from 13 to 63 (median = 34 and 37.5 for auto and allo patients respectively). Plasma levels of BU were analyzed from 15 min through 6 hrs after the first, ninth and thirteenth dose of Busulfex. Dose 1 AUC was extrapolated to infinity (AUC*inf*) and dose 9 and 13 AUC were corrected for steady state (AUC*ss*). The target AUC*inf* below 1500 (Mol-min for Busulfex dose 1 was achieved for 88 (90%) of the 98 patients with fully evaluable day 1 and day 9 pharmacokinetics representing drug exposure generally considered safe in terms of risk of VOD. This was achieved with a mean AUC*inf* for dose 1 of 1141 (Mol-min (sd 324). The mean dose 1 AUC for the autologous patients was 1144 (Mol-min (sd 222) and for the allogeneic patients was 1106 (Mol-min (sd 318). The pharmacokinetic profile of the first Busulfex dose predicted with good precision the steady-state AUC of the ninth Busulfex dose. The mean dose 9 AUC was 1225 (Mol-min (sd 216) for the autologous patients and 1167 (Mol-min (sd 224) for the allogeneic patients. All patients engrafted (ANC >0.5 x 10^9/L) with median days to engraftment for auto of 10 days (range 8 to 19) and for allo 13 days (range 9 to 29). There was no late graft failure or rejection. Freedom from progression, progression free survival, and overall survival compare favorably with comparable risk groups of oral busulfan treated patients at a median follow up of almost one year. This comparable effectiveness and improved safety profile of the Busulfex substitution for oral busulfan is presumably due to the narrow std deviation achieved with this preparation. The reduced inter patient and inter dose variability of Busulfex compared with oral busulfan will make accurate pharmacokinetic based therapy with limited sampling strategy feasible and busulfan based transplantation safer and more effective.

79

A RANDOMIZED, PROSPECTIVE COMPARISON OF ALLOGENEIC BONE MARROW (BM) AND PERIPHERAL BLOOD PROGENITOR CELL (PBPC) TRANSPLANT IN THE TREATMENT OF HEMATOLOGIC MALIGNANCIES: AN UPDATE.

AC Vigorito, AM Azevedo, KAB Eid, FJP Aranha, GB Oliveira, MEP Correa, ECM Miranda, ARC Reis, JFC Marques Jr, I Lorand-Metze, WM Azevedo and CA Souza. BMT Unit, State University of Campinas, SP, Brazil

We present updated results of a prospective, randomized study comparing both techniques with regards to engraftment, acute and chronic graft versus host disease (GVHD) and survival. Since Feb 95, 50 patients with hematologic cancers received Human Leukocyte Antigens (HLA) -identical sibling BM (group A) or PBPC (group B) transplants. Evaluable patients at the end were 24 (A) and 23 (B). Median age was 36 years (17-56) in A and 29 years (7-51) in B. Conditioning was Busulfan-Cyclophosphamide (Bu-Cy2); GVHD prophylaxis was cyclosporine-metrotrexate (CSA-MTX). PBPC were harvested after 5 days of G-CSF 10μg/g/d. The median content of CD34+ cells x10^6/Kg was 5.09 (1.1-17.5) in A and 5.75 (1.2-71.6) in B (p=0.2). Median of days for an absolute neutrophils count (ANC) >0.5 x10^9/l was 18 (13-30) in A and 15 (11-25) in B (p=0.04). Platelet counts >20 x10^9/l occurred at +19 (10-40) in A and +13 (9-36) in B (p=0.001). Median of hospital discharge was +26 (18-69) in A and +21 (16-42) in B (p=0.1). The incidence of acute GVHD of grade (2 was 21,1% (A) and 26,3% (B) (p=0.5). The incidence of chronic GVHD was 50% with BM and 61.1% with PBPC (p=0.76); extensive disease was present in 50% and 100%, respectively (p=0.01). The relapse rate was 8.3% (2/24 pts) in A and 17.3% in B (4/23 pts), at a median follow-up of 719 (106-1314) and 411 (141-1349) days, respectively. The estimates of overall survival at 1300 days are 44% and 53% respectively (p=0.64) and disease free survival, 42% and 59% (p=0.27). PBPC resulted in faster engraftment, the incidence of acute and chronic GVHD was similar but the severity of chronic GVHD remain higher with PBPC. No differences in survival were detected so far.

80

PHASE II TRIAL OF INTRAVENOUS BUSULFAN (IV BU) AND CYCLOPHOSPHAMIDE (CY) IN PEDIATRIC PATIENTS: PRELIMINARY PHARMACOKINETICS.

D.A. Well, K.W. Chan, M.R. Klemperer, R. Kadota, and M. Kletzel for the Pediatric Blood and Marrow Transplant Consortium, Saint Louis University, St. Louis, MO, M.D. Anderson Hospital, Houston, TX All Children's Hospital, St. Petersburg, FL, Children's Hospital of San Diego, CA, and Children's Memorial Hospital, Chicago, III

Oral busulfan is a well established component of preparative regimens for hematopoietic stem cell transplant (HSCT). An intravenous formulation would eliminate oral administration and first-pass hepatic clearance as variables in drug dosing; issues especially problematic in the pediatric population.

In a Phase II open-label, multi-center trial, seven pediatric patients have undergone HLA -matched sibling allogeneic HSCT after a conditioning regimen with a new formulation of IV BU (Busulfex™) and CY. IV BU was given a dose of 0.8 mg/kg based on actual body wt q6h x 16 doses, followed by CY 50 mg/kg iv q24 h x4 doses. The median age was 9y (3-15y), with median wt of 22 kg (16-49 kg). Transplants were performed for AML (2), ALL (1), MDS (1), thalassemia (2), and (-mannosidosis (1). Plasma levels of BU were analyzed after 1st and 9th doses of IV BU. If the first dose AUC did not fall within the target range of 900-1500 (Mol-min, doses 7-16 were adjusted. The median dose 1 and dose 9 AUC levels were 909 (Mol-min (range 450 to 1306) and 1169 (Mol-min (range 885-1508), respectively. No dose adjustments were needed for 3 children > 25 kg. Lower AUCs were noted for the smaller children, with 3 of 4 requiring an increase in dose. 7/7 engrafted by 12 d (range 11-14d) and no unique toxicities were noted. Two patients had mild VOD which was resolved. 5/7 are alive and well, in remission or with correction of genetic disorder at 3 - 12mo post HSCT (2 deaths - recurrent leukemia (1), GVHD(1)).

IV BU (Busulfex™) results in relable pharmacokinetics with a dose of 0.8 mg/kg in children > 25 kg, with associated engraftment and no unexpected toxicity. The study is continuing to enroll smaller children to define dosing in this group.

81

TOXICITY OF SINGLE DAILY DOSE (SDD) GENTAMICIN IN STEM CELL TRANSPLANT (SCT) RECIPIENTS.

D.I. Warkentin, C. Ippoliti, J. Bruton, K. Van Besien, and R. Champlin; Vancouver Hospital and Health Science Center, Vancouver, B.C., Canada and The University of Texas, M.D. Anderson Cancer Center, Houston, TX, U.S.A.

Published clinical trials suggest SDD aminoglycosides in combination with other antimicrobial agents are sage and effective in SCT recipients. Several months after the institution of SDD gentamicin as part of our treatment for febrile neutropenia was noted ototoxicity in several patients (pts). This finding prompted an immediate evaluation to determine incidence, and risk factors associated with the administration of SDD gentamicin. Medical records of all adult SCT recipients who received SDD gentamicin for the treatment of neutropenic fever during a two month period were evaluated. Thirty-three patients, mean age 42 years (range 19-61 years), received gentamicin 5mg/kg/day. Mean duration of therapy was 7 days (range 3-32 days). All patients received vancomycin and 17 received cisplatinum. All had normal renal and otologic function prior to therapy. Serum gentamicin levels were monitored only when renal function deteriorated. One patient (3%) developed nephrotoxicity, defined as rise in serum creatinine (2x baseline. Four patients (12%) developed clinically significant ototoxicity. All four patients had normal serum creatinine concentration before and during therapy. The mean duration of gentamicin in patients who developed ototoxicity was 20 days compared to 9 days in patients who did not experience ototoxicity (p=0.001). The mean duration of gentamicin therapy prior to the onset of symptoms was 12 days (range 3-19 days). Four patients developed decreased hearing. In only one of these patients did these auditory symptoms resolve with drug discontinuation. Two of these 4 patients also developed severe vestibular toxicity which did not resolve with drug discontinuation. SDD gentamicin was associated with clinically significant ototoxicity in 12% of our patients. One must be cognizant of the increased risk of irreversible ototoxicity when administering SDD gentamicin for a prolonged duration to adult SCT recipients.

82

ETHANOL TOXICITY WITH HIGH-DOSE ETOPOSIDE: A CASE REPORT IN TWO BONE MARROW TRANSPLANT RECIPIENTS.

JA Williams, PE Kintzel, E Abella, J Klein, R Dansey, C Karanes, R Baynes; Barabara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

This report describes an unexpected and previously unreported adverse effect of etoposide infusion in two pediatric bone marrow transplant patients. The epipodophyllotoxin etoposide is a topoisomerase II inhibitor with activity against leukemias, lymphomas, testicular tumors, neuroblastoma, sarcomas and brain tumors. It is included in several high-dose chemotherapy regimens used for autologous and allogeneic bone marrow or stem cell transplantation. Bone marrow transplantation has allowed greater therapeutic efficacy and circumvented the dose limiting myelosuppression of etoposide, permitting doses 5 to 10 times the conventional dose. Etoposide is poorly soluble in water therefore absolute alcohol is present in the formulation at a concentration of 30.5% to solubilize this lipophilic drug. These two patients received a four hour intravenous infusion of high-dose etoposide (60 mg/kg) in addition to total body irradiation as preparatory regimen for allogeneic BMT. During the fourth hour of etoposide administration, both patients had an acute alteration in mental status consisting of somnolence, agitation, and emotional lability in association with an acute metabolic acidosis. Ethanol intoxication was confirmed in the second patient with an ethanol level measured at 87 mg/dl. The predicted level of ethanol would be 165-195 mg/dL minus the amount metabolized over four hours (67-95 mg/dL). Muscular incoordination is seen with ethanol levels of <50 mg/dL, while respiratory failure, coma and death occur with ethanol levels of 400-700 mg/dL. The effects of ethanol intoxication in these pediatric patients were probably aggravated by concomitant use of the ancillary drugs diphenhydramine and lorazepam. Ethanol intoxication should be considered as a rare but potential serious side effect of high-dose etoposide therapy in pediatric patients. Consideration should be given to replacement with etoposide phosphate, a formulation which does not contain alcohol, or prolongation of etoposide infusion times with careful pharmacologic monitoring.

83

SUCCESSFUL NONMYELOABLATIVE ALLOGENEIC STEM CELL GRAFT IN A PATIENT WITH AML AND PROGRESSIVE PULMONARY ASPERGILLOSIS.

CO Xun, PA McSweeney, VC Broudy, JA Thompson and R Storb. Fred Hutchinson Cancer Research Center (FHCRC) and University of Washington, Seattle, WA

Active aspergillus infection is usually a contraindication for conventional high-dose hematopoietic stem cell transplant (HSCT) at the FHCRC given that all patients (pt) so treated in the past have died from progressive aspergillosis. Here we report a successful nonmyeloablative allogeneic HSCT as a rescue procedure in a 49 year old pt who had prolonged marrow aplasia and active pulmonary aspergillus infection after induction chemotherapy. The pt presented with AML/M1 with 85% CD13+, CD33+, CD34+ blasts in the marrow and received two cycles of induction chemotherapy (Ara-C 200mg/m^2/d 1-7 and Ida 12mg/m^2/d 1-3) due to persistent disease. His marrow became aplastic and he developed pulmonary nodular aspergillus lesions after being neutropenic with 0 neutrophils for 49 days despite treatment with amphotericin B and G-CSF. An HLA-matched nonmyeloablative HSCT was given as a rescue procedure for prolonged marrow aplasia. The pt received TBI 200 cGy on day 0 followed by 10x10^6/kg G-CSF mobilized CD34+ cells from his brother after confirmation of marrow aplasia without evidence of leukemia and neutropenia for 61 days with rapid left upper lobe consolidation of the lung due to aspergillosis. Post transplant immunosuppression included cyclosporine (CSP) day-1 to 80+ and mycophenolate mofetil (MMF) day 0-27. He engrafted with an ANC>500 by day +8 and become platelet and red cell transfusion independent by day +13. On day +14, 99% of neutrophils and 70% of lymphocytes were donor origin. The day +28 marrow exam showed 99% donor cells with no evidence of leukemia. The pt was discharged to outpatient care by 3 weeks after transplant. He is now 80 days after transplant with a stable donor graft and no evidence of GVHD on CSP. The anti-fungal treatment was changed to oral viroconazol after total dose of 2.1 gram of amphotericin B treatment. Pulmonary lesions are slowly resolving on follow-up imaging studies. In conclusion, a successful allo-HSC graft was achieved after low dose TBI and CSP/MMF to control both host vs graft and graft vs host reactions in a pt with chemo-induced prolonged marrow aplasia and progressive pulmonary aspergillosis. The transplant was associated with minimal toxicity and the pulmonary aspergillosis infection is slowly resolving. This procedure should be prospectively tested as a means to rescue pts suffering from refractory chemo-induced marrow aplasia.

84

ATG AS PART OF THE CONDITIONING IN MUD BMT-CLINICAL AND BIOLOGICAL RESULTS.

R. Schwerdtfeger, H. Baurmann, N. Bonnefoy-Berard, C. Kreißig, J.P.Revillard*; BMT Center DKD, Wiesbaden, GERMANY, *INSERM U80, Hôpital E. Herriot, Lyon, FRANCE*

Despite the increasing use of ATG for conditioning in MUD BMT little is known about its biological effects and influence on clinical outcome in this context. We present our clinical as well as the pharmacokinetic and lymphocyte (ly) reconstitution data generated in 50 consecutive MUD transplants. 34 males and 16 females, median (md) age 34y, received unmanipulated bone marrow (BM) from matched (n=43) and DRB1 mismatched (n=7) voluntary donors for CMLCP (n=20), CMLBC (n=3), high risk/relapsed AML (n=16), ALL/NHL (n=10) and SAA (n=1) resp. Thymoglobulin* was added to standard chemo/radiotherapy conditioning from d -5 to -2 at 2.5mg/kg/d (n=40), 3.75mg/kg/d (n=6) or 5mg/kg/d (n=4). 45 patients (pts) engrafted at md of 19d (14-32). 5 pts died before engraftment, 1 pt had 2nd BM failure. Acute GvHD°II-IV developed in 19 (°IV in 3), chronic GvHD in 7 pts (4 limited, 3 extended). Probability of survival is 68% in CMLCP and 34% in AL/NHL/SAA (md observation time 9.5 mo). 24 pts died from infections (n=9), relapse (n=6), treatment related toxicity (n=4), aGvHD (n=2), cGvHD, EBV LPD and BM failure (n=1 each). Rabbit IgG (ELISA) and specific anti-lymphocyte antibodies (ATG eq) quantified by flow cytometry can be detected in pts sera in decreasing concentrations up to 100d after BMT. Ly typing reveals normal NK cell reconstitution (md of 88% of ly) and prolonged depression of CD3+ and CD4+ cell counts (md of 101 and 14/(l) at d100, resp. In selected pts slowly rising CD3+ cells can only be shown after disappearance of ATG from the serum. Initially all CD3+ and a part of CD3 negative cells are coated by rabbit IgG, with decreasing overall coating from d70 onwards. In addition, decreased CD3 md fluorescence intensity compared to pretreatment values even after disappearance of ATG from the serum indicates down modulation of surface CD3/TCR possibly further contributing to immunosuppression and tolerance induction after MUD BMT. Conclusions: In vivo T cell depletion of the graft with ATG results in a high level of engraftment and low incidence of severe GvHD. Relapses so far are no more frequent than expected in this high risk population. Profound quantitative and qualitative depression of T cell function can be demonstrated during the first 100d after MUD BMT.